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PHOSPHORUS CONTENT AND BUFFER CAPACITY OF PLANT SAP AS RELATED TO THE PHYSIOLOGICAL EFFECT OF PHOSPHORUS FERTILIZERS IN FIBROUS LOW-MOOR PEAT[1]

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INTRODUCTION

Ever since the initiation of systematic fertilizer work upon the organic soils of the Everglades, distinctly inimical effects have been observed to follow the use of the more soluble forms of phosphorus under certain conditions on the growth of a considerable number of agricultural plants. In line with this experience, and following the applications of different kinds of phosphatic materials to a series of plots[3] on typical saw-grass peat, a planting of shallu (*Andropogon sorghum*) resulted in a distinctly retarded growth on those plots that had received the more soluble forms, such as the superphosphates. Plants on plots that were treated with less soluble materials, such as finely ground rock phosphate, were entirely normal in appearance, however, and much like those to which no phosphorus had been applied.

In an endeavor to ascertain the cause of these disturbances in crop development a study of the sap of the plant was undertaken. This study was extended to embrace a crop of buckwheat (*Fagopyrum esculentum*) grown on the same plots. The growth of this plant was normal on plots treated with phosphorus, but particularly abnormal where phosphorus was omitted and lime added. The same treatment of lime without phosphorus was not detrimental to shallu, however. Corresponding leaf-sap studies of sugarcane (*Saccharum officinarum*), of rape (*Brassica napus*), and of corn (*Zea mays*), grown on similarly treated plots, are also included.

MATERIALS AND METHODS

For the most part leaves only were selected for the sap extractions but in the case of buckwheat and sugarcane, stem saps also were obtained. Samples were always taken between 8 and 9 a. m., and, when leaves were used, they were removed from the same portions of the plants to insure as much uniformity as possible in the sampling procedure. Thus the leaf growing below the last upper well-defined stem internode was selected in the case of the shallu, corn, and sugarcane. The entire buckwheat plant was taken and the leaves were stripped for one sample while the stems were used for another, after discarding the leaf petioles. Samples, usually consisting of about

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[2] Thanks are due Dr. R. V. Allison, soils specialist in charge, for helpful advice during the course of the work. The writer is also indebted to Dr. A. Daane, agronomist, under whose immediate supervision the crops used in these experiments were grown.

[3] Studies on phosphorus sources were begun on these plots in 1929 by Dr. Allison.

100 leaves or stems, were obtained from the central portions of the plots to avoid border effects.

The green plant material was ground in a burr mill and immediately subjected to a pressure of 16,000 pounds per square inch in a steel receptacle. The sap so obtained was centrifuged and portions were used for electrometric titration. When the pH only of the sap was desired a quinhydrone electrode was used. For the establishment of the buffer curves a hydrogen electrode was employed, using 15 ml of the sap and N/5 solutions. Conductivity measurements were made in an Ostwald cell with a Wheatstone bridge and alternating-current galvanometer hook-up. Sap solids were determined with an Abbe refractometer as applied by Gortner and Hoffman (*6*).[4] The preparation of sap samples, outlined above, was carried through as rapidly as possible and all reaction measurements were completed within 2 to 3 hours after the sample had been collected. The remainder of of the sap was stored in flasks with toluene added as a preservative.

Total phosphorus was determined by evaporating and igniting 5 ml of the sap with magnesium nitrate for the colorimetric molybdenum blue method, amino-naphtol sulphonic acid being used for the reducing agent, as recommended by Fiske and Subbarow (*5*) and Parker and Fudge (*9*). Inorganic phosphorus was determined colorimetrically in sap clarified by the method suggested by Emmert (*4*) for crushed plant tissue. It was found necessary not only to prepare the decolorizing carbon (Norit) by leaching out traces of phosphoric acid with 1 percent H_2SO_4 by volume, but also to wash the precipitate with the diluted acid in order to make sure that there was no occlusion of phosphorus.

The plots, 18 by 100 feet in size, from which the samples used in this work were obtained, were laid out as a duplicate series for 16 different treatments of which 11 are included in this study. In the course of the experiments several different crops were growing at the same time, each planting consisting of a definite number of the 18-foot rows running across each of the 32 plots. The soil is typical of the brown fibrous peat of the Everglades, which, with cultivation, breaks down into a very dark-brown to black, fine-grained, fibrous state. In this locality it is about 7 feet deep and lies directly upon porous limerock with local pockets of marl. The reaction of this soil, undisturbed by burning, ranges between pH 5.6 and 6.0.

The basic fertilizer mixture used in these treatments was a 6–12–12 (N–P–K) combination in which the nitrogen, calculated as N, was derived equally from $NaNO_3$ and $(NH_4)_2SO_4$. The phosphorus sources are recorded below, while the potassium (K_2O) is derived from the sulphate salt. The applications were at the rate of 1,200 pounds per acre, except where colloidal phosphate and rock phosphate were used. In these instances 2 and 4 times the amount of phosphorus (P_2O_5) were added, respectively. Applications were made to these plots on this basis in March 1929, March 1930, April 1931, and September 1932. A general treatment with copper, manganese, and zinc in the form of their sulphates at the rate of 50, 50, and 12½ pounds per acre, respectively, was used upon all plots in accordance with the earlier findings of Allison et al. (*1*). These were included in the first

[4] Reference is made by number (italic) to Literature Cited, p. 300.

application only. The treatments made upon the plots included in this study are as follows:

Plot no.	Treatment
1	Check (Cu+Mn+Zn, only, as in all others).
2	K (from sulphate of potash).
3	N+K (from $(NH_4)_2SO_4$ and $NaNO_3$).
4	N+P+K (16-percent superphosphate).
5	N+P+K (45-percent superphosphate).
6	N+4P+K (raw rock phosphate).
8	N+2P+K (colloidal phosphate).
9	Check (Cu+Mn+Zn, only, as in all others).
13	N+K+lime.
15	N+P+K+lime (16-percent superphosphate).
16	N+P+K+S (16-percent superphosphate).

Aside from the materials regularly used in the fertilizer mixture, lime was added at the rate of 2,000 pounds per acre; colloidal phosphate at 1,090 pounds; raw rock phosphate, finely ground, at 2,400 pounds; and sulphur at 300 pounds per acre, respectively. The first three of these materials were applied broadcast, while sulphur was incorporated in the fertilizer mixture, drilled in the row with it and worked in. Seven crops had been removed from these plots previous to those under discussion in this paper.

EXPERIMENTAL DATA

RESULTS WITH SHALLU

In the growth of a crop of shallu planted October 8, 1931, a marked physiological disturbance was observed on plots 4 and 5, and especially on plot 16. The first cutting was made on January 14, 1932. This injury consisted of a general stunting of the plants, accompanied by a chlorotic appearance and the inability to tassel and produce seed (fig. 1).

Since the trouble appeared to be associated with the application of soluble phosphates, leaf samples were obtained from plants on plots that represented the various phosphatic treatments. Table 1 gives the results obtained from these samples which were taken on March 19 when the seeds of the plants were in the milk stage. The data show that, whereas there was a moderate increase over the check in sap phosphorus in the leaves from the colloidal phosphate treatment of plot 8, there was a considerably greater increase for the 16-percent superphosphate treatment of plot 4. The amount of phosphorus in the leaf sap of plants from plot 16, to which sulphur was added, was still greater. In contrast, the content of phosphorus in the sap of leaves of shallu plants from the lime plus phosphate plot 15 was but little higher than that from the check plot 2 receiving potash but no phosphate. Characteristics of the saps of plants upon plot 1 must be considered in a special way in this and in subsequent treatments because the lack of potassium in the fertilizer caused the crops to be almost complete failures upon these checks.

Since the increase in total acidity tended to follow that of the total phosphorus (table 1), and since the pH values were but little affected, it would seem that the phosphorus taken up by the plant was exerting a strong buffering action. Where lime was added with the soluble phosphate on plot 15 both growth injury and sap phosphorus were

TABLE 1.—*Active and potential acidities and total phosphorus concentrations of leaf saps of shallu from plots upon which different fertilizer treatments were used, Mar. 19, 1932*

Plot no.	pH	Acidity [1]	Total P_2O_5	Plot no.	pH	Acidity [1]	Total P_2O_5
		Cc	*P. p. m.*			*Cc*	*P. p. m.*
1	5.80	2.55	1,909	8	5.87	2.25	2,114
2	5.39	1.85	1,713	15	5.54	2.40	2,053
4	5.71	2.75	2,457	16	5.59	5.20	6,471

[1] Acidity values are cubic centimeters of N/10 NaOH per 5 cc of sap.

reduced. Where the lime was replaced by sulphur in plot 16, plant growth was most seriously retarded. A correlation was particularly evident between growth injury and soluble phosphorus in this instance.

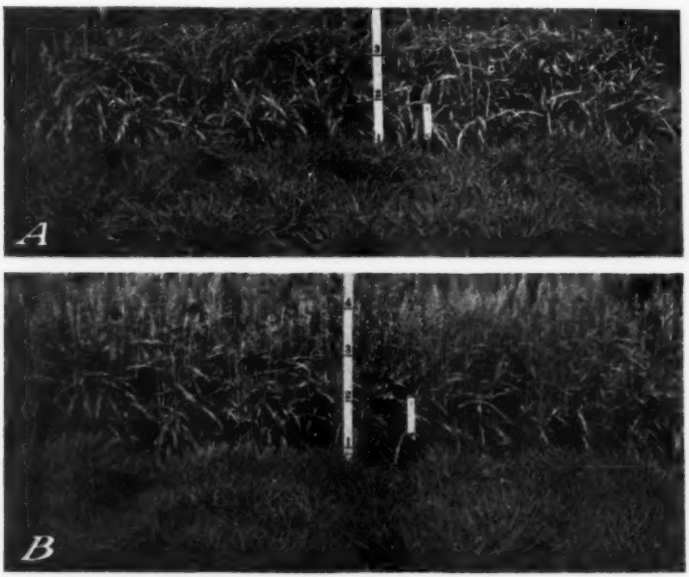

FIGURE 1.—Shallu at the March 19 sampling: *A*, growth on plot 5 was decidedly retarded because of the inclusion of soluble phosphate in the fertilizer mixture; *B*, phosphorus was omitted for plot 2, where entirely normal growth was obtained.

The subsequent growth of the second ratoon crop of shallu exhibited appreciably less injury. Leaf samples taken on June 1 at the same stage of growth as those of the previous sampling on March 19 again contained more sap phosphorus when soluble phosphates had been applied as on plots 4 and 16. For plants from plot 4 the amounts were (tables 1 and 2) 2,457 and 2,728 p. p. m. as P_2O_5 and for plot 16 they were 6,471 and 3,824 p. p. m., respectively, for the crops of March 19 and of June 1. Figure 2 shows that almost all of the sap phosphorus was inorganic in nature and that it varied with total acidity. The pH values were practically constant for all of the treatments.

TABLE 2.—*Hydrogen ion concentration, acidity, phosphorus, soluble solids, and conductivity of the expressed leaf saps of corn, shallu, and sugarcane from plots upon which different fertilizer treatments were used, June 1 to 6, 1932*

Crop and plot no.—	pH	Acidity [1]	Total P_2O_5	Inorganic P_2O_5	Soluble solids	Conductivity [2]
		Cc	*P. p. m.*	*P. p. m.*	*Percent*	*Mhos*
Shallu:						
1	5.37	2.60	2,597	2,643	10.53	228
2	5.67	2.35	2,151	1,951	9.43	218
4	5.59	3.00	2,728	2,632	10.13	238
5	5.56	3.20	3,109	3,290	10.83	236
6	5.68	2.70	2,667	2,632	12.03	239
8	5.63	2.20	2,113	2,547		236
15	5.60	2.35	2,057	2,168	9.43	230
16	5.66	3.60	3,824	3,871	9.33	221
Corn:						
1	5.63	2.60	985	1,013	10.53	255
2	5.34	3.35	938	880	9.43	263
4	5.51	5.50	1,882	1,786	11.33	258
6	5.73	2.90	2,326	2,300	11.93	240
15	5.59	3.10	1,558	1,536	9.63	245
16	5.51	4.85	2,770	2,667	9.33	234
Sugarcane:						
1	5.37	1.75	1,367	1,419	5.53	247
2	5.46	1.40	1,158	1,227	5.43	269
4	5.29	2.45	2,358	2,372	5.45	309
6	5.68	1.35	1,500	1,456	5.33	301
15	5.54	1.50	1,588	1,551	5.36	287
16	5.54	2.30	2,344	2,620	5.23	297

[1] Acidity values are cubic centimeters of N/10 NaOH per 5 cc of sap.
[2] Conductivity values equal reciprocal ohms × 10^{-4} at 28° C.

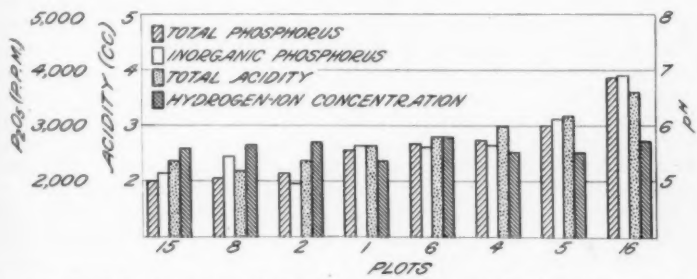

FIGURE 2.—Total phosphorus, inorganic phosphorus, total acidity, and pH values of leaf saps of shallu harvested from variously tested plots June 1. The soil treatments are given in the tabulation on page 289.

The third ratoon crop of the shallu was removed September 20. No physiological disturbances were in evidence in the growth of this crop, but the appearance of a leaf-spot disease at the tasseling stage prohibited a definite comparative study of the leaf saps. The phosphorus content of the saps was about the same for all the plots (table 3) and was about as high as that of the harvest of March 19 when growth was so seriously retarded. This point will be discussed later.

RESULTS WITH BUCKWHEAT

A reapplication of fertilizers was made on the plots under study and a crop of buckwheat was planted November 25. Instead of exhibiting injury on plots 4, 5, and 16 where the soluble phosphorus had been added, as was the case with shallu, buckwheat showed a slightly improved growth. Moreover the growth on plot 13 with lime, but without phosphorus, was almost a complete failure.

TABLE 3.—*Leaf-sap analyses of shallu from plots upon which different fertilizer treatments were used, September 20, 1932*

Plot no.—	pH	Acidity [1]	Total P_2O_5	Soluble solids	Amount expressed [2]	Conductivity [3]	Total dry matter
		Cc	*P. p. m.*	*Percent*	*Percent*	*Mhos.*	*Percent*
1	5.55	2.00	1,990	9.1	37.1	126	26.80
2	5.10	2.50	2,048	8.7	28.4	136	34.73
4	5.80	2.40	2,335	12.0	34.4	129	30.47
8	5.81	1.70	2,326	11.3	27.6	132	31.06
15	5.87	1.75	2,128	12.8	31.8	120	32.47
16	5.83	3.60	.....	13.6	34.8	136	31.53

[1] Acidity values are cubic centimeters of N/10 NaOH per 5 cc of sap.
[2] Amount expressed per weight of green material.
[3] Conductivity values equal reciprocal ohms $\times 10^{-4}$ at 28° C.

Since the physiological reaction of buckwheat to these treatments was in striking contrast to that of the preceding crop of shallu, similar sap studies were made with this crop. The data for leaf saps, as shown in table 4, indicate that the changes in total phosphorus, total acidity, and inorganic phosphorus tend to parallel one another and show marked increases for the plants from the soluble-phosphate plots. Leaf sap from the plants of the limed plot 13, which received no phosphate treatment, was the lowest in phosphorus and was also very low in total acidity. This would seem to indicate that the poor growth on this plot might be due, in part at least, to a lack of phosphorus. A manganese deficiency may have developed also. In this connection it is to be observed that the saps of the buckwheat leaves from nonphosphate plots (table 4) were much lower in phosphate than the leaf saps of shallu from the same plots. This contrast is not in evidence, however, in saps from the plants of the phosphate-treated plots.

The pH values of the leaf saps of buckwheat (table 4) did not vary appreciably in passing from the nonphosphate to the phosphate plots. Electrometric titrations of these saps were carried sufficiently far to construct the titration curves shown in figure 3, *A*. The sap of leaves from phosphate-treated plot 5 was buffered considerably more than that of leaves from the nonphosphate plot 2 between the pH range of 5.5 and 11.0.

TABLE 4.—*Leaf- and stem-sap analyses of buckwheat from plots upon which different fertilizer treatments were used, Jan. 11, 1933*

Sap and plot no.	pH	Acidity [1]	Total P_2O_5	Inorganic P_2O_5	Conductivity [2]	Total dry matter
Leaf saps:		*Cc*	*P. p. m.*	*P. p. m.*	*Mhos.*	*Percent*
2	5.52	1.0	528	.....	100	16.37
3	5.54	.5	791	.....	.....	13.27
4	5.62	2.1	2,395	1,807	.....	13.97
5	5.55	2.0	2,170	1,929	92	14.60
6	5.89	1.1	925	984	.....	14.29
13	5.85	.6	502	.....	.....	14.62
15	5.71	.9	1,374	1,310	.....	15.62
16	5.60	1.3	2,182	2,041	.....	14.14
Stem saps:						
2	4.80	2.0	545	548	137	15.60
3	4.70	1.3	553	534	.....	13.81
4	4.75	2.7	3,097	2,930	.....	14.21
5	4.80	2.4	3,309	3,604	149	14.20
6	4.92	2.0	1,750	.....	.....	.....
13	4.75	.9	600	580	.....	14.93
15	5.02	2.1	2,544	2,694	.....	16.17
16	4.78	2.9	3,275	3,478	.....	15.27

[1] Acidity values are cubic centimeter of N/10 NaOH per 5 cc of sap.
[2] Conductivity values equal reciprocal ohms $\times 10^{-4}$ at 28° C.

A somewhat similar variation in buffer capacity is in evidence for the saps of the stems from both plots 2 and 5 (fig. 3, *B*). The initial hydrogen-ion concentrations of these saps were distinctly higher than those from the leaves (fig. 3, *A*), however, and they were also more strongly buffered in the lower hydrogen-ion concentration ranges. The buffer capacities of the saps in the higher hydrogen-ion concentrations were about the same for both leaf and stem.

The total phosphorus content of the stem saps was found to be greater than that of the leaves, the minimum and maximum values being, for the stem saps, 545 and 3,309 p. p. m., and for the leaf saps, 528 and 2,395 p. p. m. (table 4). For both leaves and stems the total acidities and inorganic phosphates tend to vary rather closely with the corresponding content of total phosphate. Saps of buckwheat plants from the soluble-phosphate plots were about as high in phosphorus as those of the shallu from the same plots.

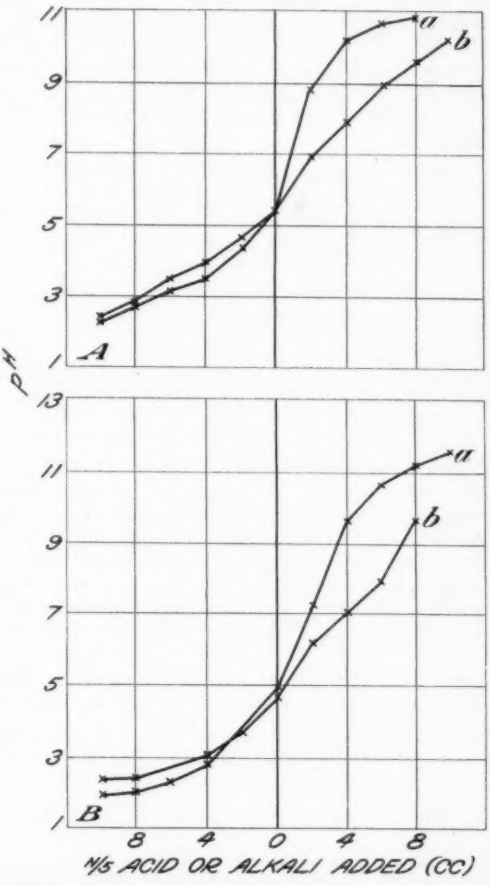

FIGURE 3.—Titration curves of 15 ml of leaf sap (*A*) and stem sap (*B*) of buckwheat plants, January 11, 1933. Plot 2 (*a*) received sulphate of potash only and plot 5 (*b*) received nitrates, sulphate of potash, and 45-percent superphosphate.

BUCKWHEAT VERSUS SHALLU

Following buckwheat the plots were planted to peas, after which shallu was again seeded on April 28, 1933. Characteristic growth disturbances on the soluble phosphate plots 4, 5, and 16 were again noted, except that they were not as severe as those of the March 19, 1932, harvest, discussed above. Titration curves of leaf saps of plants from plots 2 and 5 (fig. 4, *A*) show that the sap of plants from the nonphosphate plot 2 was naturally well buffered and that the addition of

soluble phosphorus on plot 5 increased the buffer effect within the range pH 5.0 to above 9.

Figure 4, *B*, shows that the buffer capacity of buckwheat leaf sap is much less than that of shallu. Comparison of figures 3, *A*, and 4, *A*, further indicates that the addition of a soluble phosphate to plot 5 increased the buffer capacity of buckwheat and shallu leaf saps to about the same degree. The total phosphorus in the saps of the shallu leaves was 1,664 and 3,498 p. p. m. (table 5), respectively, for plots 2 and 5. Values from the same plots for the leaf saps of the previous buckwheat crop were 528 and 2,170, respectively (table 4). Further discussion of these points will be taken up in a later section.

TABLE 5.—*Leaf-sap analyses of shallu from plots upon which different fertilizer treatments were used, July 5, 1933*

Plot no.	pH	Acidity [1]	Total P_2O_5	Conductivity [2]	Total dry matter	Plot no.	pH	Acidity [1]	Total P_2O_5	Conductivity [2]	Total dry matter
		Cc	*P. p. m.*	*Mhos.*	*Percent*			*Cc*	*P. p. m.*	*Mhos.*	*Percent*
2	5.05	1.50	1,664	149	28.88	5	4.98	2.70	3,498	135	26.48
3	5.35	1.35	1,941	130	24.84	13	5.40	1.30	1,266	160	27.59
4	5.59	2.00	2,222	120	22.25	16	5.10	2.35	3,489	131	21.86

[1] Acidity values are cubic centimeter of N/10 NaOH per 5 cc of sap.
[2] Conductivity values equal reciprocal ohms $\times 10^{-4}$ at 28° C.

RESULTS WITH SUGARCANE

Sugarcane of the variety P. O. J. 2725 was planted in April 1931, on plots receiving the treatments outlined above. Table 2 gives the leaf-sap analysis for the sampling made June 1, while table 6 gives analyses for the sampling of August 29 from the same crop. It is to be noted that the sap phosphorus was largely inorganic in nature and that it was considerably higher in leaves from the soluble-phosphate plots 4 and 16. Total acidity values tend to follow those of total phosphorus but do not show a general increase corresponding to the increase in phosphorus in the sap of the more mature leaves of the sampling of August 29. Growth disturbances were distinctly in evidence on plot 16 and to a lesser extent on plot 4.

TABLE 6.—*Leaf- and stem-sap analyses of sugarcane, P. O. J. 2725, from plots upon which different fertilizer treatments were used, Aug. 29, 1932*

Sap and plot no.	pH	Acidity [1]	Total P_2O_5	Soluble solids	Soluble solids per 100 g tissue	Conductivity [2]	Total dry matter
Leaves:		*Cc*	*P. p. m.*	*Percent*	*Cc*	*Mhos*	*Percent*
1	5.60	1.75	3,670	6.16	55.3	178	28.56
2	5.47	1.50	3,100	6.74	55.8	178	27.92
4	5.48	2.45	4,651	6.16	58.7	177	25.00
6	5.72	2.70	3,947	6.14	55.5	182	28.24
15	5.46	1.75	3,658	6.44	51.2	176	27.96
16	5.48	2.95	5,608	6.24	53.5	180	25.12
Stems:							
1	6.56	.70	320	9.14	83.0	48	15.94
2	5.96	.20	465	9.74	82.0	47	16.80
4	5.79	.70	1,325	8.74	80.8	53	17.20
6	6.09	.40	474	8.74	81.3	62	17.11
15	5.95	.30	778	10.04	83.5	48	17.70
16	5.54	.90	1,633	9.54	81.5	64	17.75

[1] Acidity values are cubic centimeters of N/10 NaOH per 5 cc of sap.
[2] Conductivity values equal reciprocal ohms $\times 10^{-4}$ at 28° C.

It is of interest to note that while the stem sap of this variety of sugarcane was found to be much lower in phosphates and in total acidity than that of the leaf (table 6), treatment with soluble phosphates caused a striking increase in these two factors. This marked effect of soil treatment with soluble phosphates upon the concentration of this element in the stem saps of sugarcane was repeatedly observed in other experiments dealing with milling quality.

RESULTS WITH CORN

On February 2, 1932, corn was planted on the entire plot series involving the phosphate sources. Analytical results for leaf samples taken June 4 are recorded in table 2. Corn differed from the other crops studied in that it took up phosphorus about equally well whether this material was applied in the soluble superphosphate form as in plot 4, or as finely ground raw rock phosphate as in plot 6. Lime again depressed the phosphorus content of the sap of the plant (plot 15), while sulphur increased it (plot 16). Results with corn were similar to those with buckwheat in that the absence of a phosphatic fertilizer (plot 2) caused a marked reduction in sap phosphorus. Corn grew well on plot 13 to which lime and potash but no phosphorus was added, under which conditions buckwheat failed almost completely.

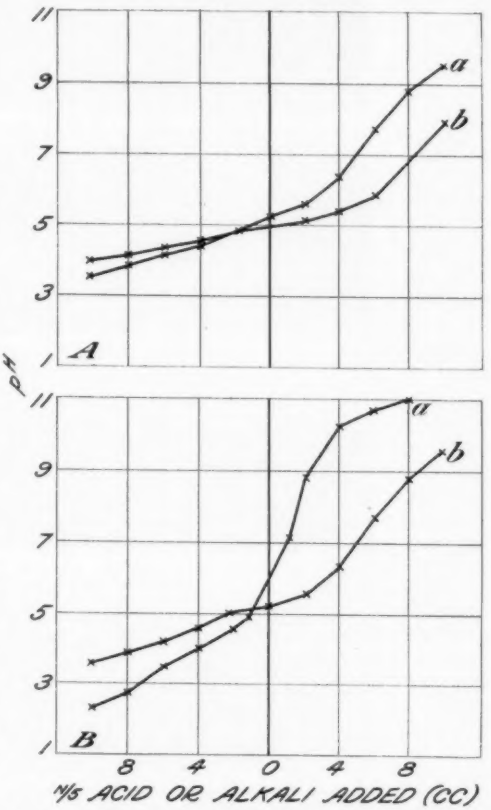

FIGURE 4.—Titration curves of 15 ml of leaf sap from shallu (*A*) and from buckwheat (*B*). Plot 2 (*a*) received sulphate of potash only and plot 5 (*b*) received nitrates, sulphate of potash, and 45 percent superphosphate.

RESULTS WITH RAPE

Rape was planted on the entire plot series on December 18, 1932. Marked response to phosphate was shown early in the growth of this plant as recorded in figure 5. Unfortunately buffer-curve data were not obtained but the comparative pH, acidity, and phosphate values for the leaf saps (table 7) indicate that they were in marked contrast with one another, possibly more so than for any of the other crops.

FIGURE 5.—Growth of rape, December 23, on plots all of which received nitrates and potash with the phosphate source varied as shown on page 289: *A*, Plot 3; *B*, plot 4; *C*, plot 13; *D*, plot 15; *E*, plot 16.

TABLE 7.—*Leaf-sap analyses of rape from plots upon which different fertilizer treatments were used, Dec. 23, 1932*

Plot no.	pH	Acidity [1]	Total P_2O_5	Conductivity [2]	Total dry matter	Plot no.	pH	Acidity [1]	Total P_2O_5	Conductivity [2]	Total dry matter
		Cc	*P. p. m.*	*Mhos*	*Percent*			*Cc*	*P. p. m.*	*Mhos*	*Percent*
1	6.37	0.65	346	106	10.82	13	6.51	0.25	370	138	9.82
3	6.40	.50	385	142	11.15	15	6.17	.70	764	125	9.01
4	6.00	.75	1,961	135	9.99	16	6.07	1.00	2,214	135	9.13
6	6.39	.50	560	133	9.14						

[1] Acidity values are cubic centimeters of N/10 NaOH per 5 cc of sap.
[2] Conductivity values equal reciprocal ohms × 10^{-4} at 28° C.

Table 7 shows that the content of phosphorus was low in the sap of rape plants grown under the nonphosphate plots 3 and 13, and that it was from 5 to 6 times greater in those of the phosphate plots 4 and 16. Increases in plant growth in response to soil treatment with phosphate materials also were striking (fig. 5). The effect of lime was very marked in depressing the phosphate concentration of soluble phosphorus in the sap of the plants (table 7), and caused a near failure of growth on plot 13 which received lime alone without phosphorus treatment of any kind.

SOLUBLE PHOSPHORUS IN PLANTS VERSUS THE PHYSIOLOGICAL EFFECTS OF PHOSPHATE ADDITIONS

Since the growth of shallu and, to a lesser extent, of corn and sugarcane was adversely affected by the application of soluble phosphates whereas buckwheat and rape were distinctly favored by the same treatments, it is of interest to compare certain characteristics of the saps of these plants in an effort to develop some reasonable explanation for the observed differences in response. Perhaps the outstanding sap variation in this connection is that of phosphorus content, and, for purposes of comparison, these values are grouped in table 8. As might be expected, the leaves of shallu, corn, and sugarcane, being less succulent than those of rape and buckwheat, have a higher content of soluble phosphorus. In a similar comparison of plants from plots 2 and 4, untreated and treated with superphoshate, respectively, it is seen that the content of sap phosphorus in the rape and buckwheat was increased nearly 400 percent, whereas in the shallu, corn and sugarcane the increase averaged less than 50 percent (fig. 6).

TABLE 8.—*Sap phosphorus concentration of the leaves and stems of various crops grown on plots fertilized in the same way for each crop*

Plot no.	Shallu		Corn, June 1, leaves	Sugarcane			Rape, Dec. 23, leaves	Buckwheat	
	June 1, leaves	Sept. 20, leaves		June 1, leaves	Aug. 29			Jan. 11	
					Leaves	Stems		Leaves	Stems
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
2	2,151	2,048	938	1,158	3,100	465	385	528	545
4	2,728	2,335	1,882	2,358	4,651	1,325	1,961	2,395	3,097
6	2,667	.....	2,326	1,500	3,947	474	560	925	1,750
15	2,057	2,128	1,559	1,588	3,658	778	764	1,374	2,544
16	3,824	.....	2,770	2,344	5,608	1,633	2,214	2,182	3,275

V 5 1 4 A U G 1 5 3 5 XU

It is also to be observed (fig. 6) that phosphorus concentrations run highest in shallu, the crop most sensitive to an oversupply of phosphorus, while the less sensitive crops, corn and sugarcane, come next in order in this respect. Rape and buckwheat showed the greatest phosphorus responses and had the greatest increases in the phosphorus of the expressed saps. The graphs for all crops are closest together in soluble phosphate for plots 4 and 16, where not only the best growth response was obtained in some but most serious injury occurred in others. From these results it would seem that different plant species may require approximately the same concentration of soluble phosphorus in their saps and that the optimum concentration is also rather near the point of physiological disturbance. Thus, rape and buckwheat might also be adversely affected by this relation in case they assimilated too much phosphorus, a condition that might arise in a more acid soil.

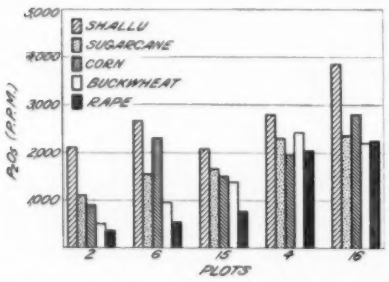

FIGURE 6.—Phosphorus concentration in the leaf saps of shallu June 1; sugarcane June 1; corn June 1; buckwheat January 11; and rape December 23. All plots received potash and all received nitrogen except plot 2. The phosphate source varied as shown in the tabulation on page 289.

SOLUBLE CONCENTRATIONS OF PHOSPHORUS IN PLANTS VERSUS SPECIFIC CONDUCTIVITIES AND TOTAL SOLIDS OF THEIR SAPS

As recorded in tables 2 to 7, analyses of the fresh saps of the crops discussed above also embraced determinations of their specific conductivities. Inspection of these data fails to show any particular relation to phosphate content. This is probably due not only to the contributory influences of other ions, such as Cl, Na, K, Ca, and SO_4, but also to the comparatively low degree of ionization of organic and phosphoric acid radicals.

For a given series of samples the total soluble solids of the saps (tables 2, 3, and 6) varied somewhat but only in an irregular manner. While certain consistent variations occurred as between different kinds of plants and between leaves and stems of the same plants it does not appear that these variations bear any significant relation to the physiological problems under discussion.

DISCUSSION

The assimilation of phosphorus tended to increase very appreciably the total but not the active acidity of the plant saps studied. The buffer capacities of plant saps and the corresponding influence of phosphates have been dealt with from many angles and are reported in a literature much too extensive to be reviewed here. An excellent review of this subject is included in a recent paper by Hurd-Karrer (*8*). Considering the necessity for ample amounts of phosphorus for plant growth, it is fortunate that phosphate solutions are so well buffered, since only slight changes in pH are possible without causing physiological disturbances. In the present instance, in fact, injury occurred without much change in pH, but rather in the presence of large increases in total acidity and soluble phosphorus. It is signif-

icant to note that almost all of the sap phosphorus was inorganic, as characterized by the presence of the phosphate radical.

As many investigators have pointed out, some plants are able to obtain a sufficient amount of phosphorus for optimum growth from a medium in which other varieties would find insufficient amounts of that element. Under the conditions of the present experiments shallu, corn, and sugarcane are in the first group, since they did not respond to soil treatments with phosphatic materials of any kind, while rape and buckwheat belong to the second group. It is also of interest that the added phosphate caused rape to have a larger growth increase and a considerably greater concentration of phosphorus in the sap than was observed for any of the other crops of this study. Alway, Shaw, and Methley (*3*) found that increase in the phosphorus content of rape due to fertilization with this element was greater than occurred in any of various crops grown in Minnesota peat soil. Hall (*7*) has reported that the rutabaga, which belongs to the same genus (*Brassica*) as rape, is a sensitive indicator crop for the need of phosphorus in English soils.

The concentration of phosphorus in the plant saps of the first group, mentioned above, tended to run much higher than in those of the second group when comparisons are made upon plant materials taken from plots to which phosphorus had not been added in any form. Since phosphorus concentrations in the saps of both groups tended to approach each other in the presence of available soil phosphate, it would appear that the optimum concentration for growth is approximately the same for all plants studied. And, since some of the crops, such as shallu, were retarded in growth in the presence of certain of the more soluble phosphate carriers, it would also seem that the point of toxic concentration of this element, when assimilated into the plant, is not far above that of the optimum for normal plant development.

It is perhaps premature to suggest that the presence of high concentrations of soluble inorganic phosphorus in the saps of these plants was entirely responsible for the growth disturbances under discussion. Almost all of the vast amount of literature relative to phosphate fertilizers mention either a positive plant response or none at all. Alway, McMiller, and Rost (*2*) found, however, that the addition of phosphate alone to a certain type of peat soil resulted not only in no benefit to the growth of any of the crops studied, but actually brought about decreases in the case of corn, flax, and sunflowers. However, phosphate combined with potash increased the yields more than potash alone. It is significant to note that this depressing effect of phosphorus, referred to by Alway, occurred in a peat soil but of a type quite different from that under consideration in this paper. Shive (*10*) also reports a condition of malnutrition in which monobasic phosphates were, under certain conditions, toxic to soybeans grown in either soil or solution cultures.

The physiological disturbances that were observed in certain of the plants used in these studies appeared to be more severe at some periods than at others. Thus shallu grew more normally at certain seasons when its sap contained as much soluble phosphorus as was found at other times when more serious injury developed. It is thought possible that this inimical effect of soluble forms of phosphorus may be more or less associated with rainfall and soil-moisture conditions. Relations of this type are under observation, and work

is being continued on the phosphorus response of plants growing in organic soils.

SUMMARY

Fresh-sap studies were made of the leaves, and in some cases of the stems, of five different plants growing under field conditions on the characteristic brown fibrous peat of the Everglades. The plots were fertilized in a systematic manner, the most important variable being the carrier of the phosphorus.

The total soluble phosphorus of the saps of these plants was much increased by soil dressings with soluble phosphates. Lime acted to reduce and sulphur to increase the assimilation of phosphorus in all plants studied. Total acidity and the total amount of inorganic phosphorus tended to vary directly with the concentration of soluble phosphorus in the sap. Active acidity of the plant sap, expressed as pH values, changed inappreciably as a result of different phosphatic treatments, whereas the buffer capacities were much greater in the saps of high phosphorus content. Neither the specific conductivities nor the total soluble solids of the saps appeared to have any definite relation either to phosphorus concentration or to physiological injury.

The crops that responded unfavorably, if at all, to phosphorus were those of which the saps were relatively high in phosphorus in the absence of soil treatment with a phosphate carrier. Those that responded favorably, on the other hand, contained sap relatively low in phosphorus.

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COMPOSITION OF THE LEAVES AND STALKS OF BARLEY AT SUCCESSIVE STAGES OF GROWTH, WITH SPECIAL REFERENCE TO THE FORMATION OF LIGNIN[1]

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INTRODUCTION

In two previous publications (*5, 27*)[3] from the Bureau of Chemistry and Soils, results were presented on the lignin, methoxyl, cellulose, ash, and silica content of the leaves and stalks of lodged and unlodged wheat plants harvested at various stages of growth. The results indicated that the percentage of lignin increased with the age of the plant and that the stalks of the lodged plants in every case contained a higher percentage of lignin than the stalks of the unlodged plants. Because of the general interest in the chemistry of lignin from annual plants, particularly from cereal plants, it seemed advisable to make a more extensive study of the formation of lignin by the plant and to determine, if possible, its interrelationship with other plant components. An opportunity for making such a study was presented when Merritt N. Pope, of the Bureau of Plant Industry, placed at the writers' disposal samples of barley plants harvested daily from the time the plants emerged until maturity. The samples selected for analysis represented for the most part weekly intervals in the development of the plant. At certain stages in the growth of the plant, samples were taken at more frequent intervals.

The present paper deals with a study of the composition of the leaves and stalks of barley at successive stages of growth. Inasmuch as the primary interest in this investigation was the development of lignin in the plant, it seemed best to confine the study to an examination of the several components of the stalk and leaves.

REVIEW OF LITERATURE

Lawes and Gilbert (*17*) in 1884 analyzed samples of wheat plants taken at different stages of development. They determined the percentages of dry matter, ash, and nitrogen, and found that the percentage of ash decreased steadily as the plants grew older.

Kedzie (*14*) in 1893 reported that the higher the percentage of crude protein of wheat straw, the lower the percentage of crude fiber and the higher its feed value. As the term "crude fiber" includes a heterogeneous group of chemical substances, his results are difficult to interpret in terms of definite chemical components.

Shaw and Wright (*32*) studied the chemical composition of sunflower and corn plants at different stages of growth. The percentages of reducing and nonreducing sugars in the sunflower declined some-

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[2] The writers express their thanks to Merritt N. Pope, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for his kindness in supplying the barley plants used in this investigation.
[3] Reference is made by number (italic) to Literature Cited, p. 318.

what irregularly but persistently during the growth of the plant. The percentages of reducing and nonreducing sugars in the corn plant in the early stages of growth were found to vary somewhat, but with a marked upward trend. However, a sudden drop occurred when the kernels were maturing. The percentage of total protein of the sunflower and corn plants decreased as the plants matured.

Ver Hulst, Peterson, and Fred (*33*) found that the actual weight as well as the percentage of "pentosans" of the corn plant increased as the plant matured. These authors determined the percentage of "pentosans" in the conventional manner, whereby the total furfural obtained when the plant material is distilled with 12 percent hydrochloric acid is calculated as percent pentosans. However, uronic acids and Cross and Bevan cellulose also yield furfural when distilled with acid, so that the values reported by these investigators include several furfural-yielding components calculated as pentosans.

Beckmann, Liesche, and Lehmann (*2*) determined the lignin content in the leaves and stalks of the rye plant at different stages of growth. Although the method they employed for estimating the percentage of lignin is far from satisfactory, their results indicate that the percentage of lignin and the percentage of methoyxl in the lignin increased as the plant matured.

Dustman and Shriver (*7*) reported on the chemical composition of *Ambrosia trifida* (giant ragweed) harvested at different stages of growth. The analytical methods were the conventional ones employed in feed-control work. They found that during blooming time, the percentages of crude protein and nitrogen-free extract were the highest. In the later stages the percentage of crude fiber increased. The percentage of pentosan increased as the plant matured.

Phillips, Davidson, and Weihe (*27*) determined the percentage of lignin, methoxyl, cellulose, methoxyl in the lignin, nitrogen, ash, and silica in the leaves and stalks of lodged and unlodged wheat plants harvested at various stages of growth. Their results indicate that the percentages of methoxyl, lignin, cellulose, and silica in the stalks increased with the age of the plant, whereas the percentages of total nitrogen and ash decreased. The percentages of methoxyl in the lignin from the lodged and unlodged stalks increased at first, but decreased slightly as the plants matured.

Malhotra (*18, 19*) determined the percentages of moisture, petroleum ether extractives, ash, sugar, starch, hemicelluloses, and total nitrogen in hard winter wheat harvested at successive periods of growth. The percentage of petroleum ether extractives was at maximum during maturity. The percentage of ash was high in the early stages of the development of the plant, then decreased. The percentage of sugar was at a minimum at the beginning; it increased later and finally decreased again. The percentage of the hemicelluloses (determined by hydrolyzing the plant material with 2.5 percent of hydrochloric acid for 4 hours and then determining the reducing sugars formed) increased somewhat during the winter months, then rose rapidly in the spring and finally decreased as the plant matured. Malhotra's results in the hemicellulose content have been criticized by Norman (*21*) on the ground that the analytical method employed is not specific for this class of substances. The percentage of total nitrogen was lower at first, but it increased during the later stages. It is not

clear, however, whether the figures represent the percentage of nitrogen in the entire plant or only in the straw. Cellulose, pentosans, and lignin were not determined, although these three components constitute the largest percentage of the organic matter of the stalks, particularly at or near maturity.

Norman (*21*) analyzed barley plants at various stages of growth. The percentages of ash and crude protein showed an initial increase, followed by a steady fall as development proceeded. The percentage of Cross and Bevan cellulose and the percentage of total furfural increased as the plant matured. The percentage of pentose (calculated as arabinose) was irregular, but was lower in the mature plant than in the young plant. The percentage of lignin increased steadily until the last two sampling periods, and then decreased, presumably because of the increased weight of the grain. Norman's figures on the percentage of lignin differ from those recorded in this paper, chiefly owing to the fact that different methods were employed for the quantitative estimation of lignin. These lignin values will be discussed later in this paper, when a comparison is made between the results obtained in this investigation and those recorded by Norman.

There has been considerable speculation concerning the nature of the precursors of lignin. Cross and Bevan (*4, pp. 177–181*), König and Rump (*16, p. 83*), and more recently, Fuchs (*10, 11*) have suggested that cellulose is the parent substance from which lignin is formed. Other investigators, such as Klason (*15*) and Rassow and Zschenderlein (*29*) have advanced the hypothesis that lignin is formed by the plant from pentoses or pentosans. The two investigators last mentioned found that plant substances high in lignin were low in pentosans, and vice versa. The possibility that soluble carbohydrates, pentoses, methylpentoses, and hexoses may be used by the plant in the formation of lignin has been suggested by Schrauth (*31*), Von Euler (*9*), and Oden (*22*).

Candlin and Schryver (*3*) have pointed out that lignified tissues contain lignin and hemicelluloses in relatively large amounts with only traces (if any) of pectins. Nonlignified tissues, on the other hand, contain relatively large amounts of pectin, small amounts of hemicelluloses, and no lignin. They failed, however, to find any direct relationship between pectin and lignin, although by the treatment of pectin with alkali they obtained a product having properties similar to those of the hemicelluloses. More recently, Ehrlich (*8*) has put forward the hypothesis that pectin is the precursor of lignin. He isolated a fraction from hydropectin which resembled lignin in certain respects; it contained methoxyl to the extent of 11.6 percent, and its percentages of carbon and hydrogen were of the same general order of magnitude as those recorded for lignin. He assumes that enzymatic and chemical reactions take place during the development of the plant to maturity, bringing about the conversion of pectin into lignin.

MATERIAL AND METHODS OF ANALYSIS

The barley plants (*Hordeum distichon palmella*, subvariety Hannchen) used in this investigation were grown under irrigation at Aberdeen, Idaho. The barley seeds were sown on May 9, and the plants emerged on May 19. The age of the plants was determined from

the day they emerged. Samples were taken daily between 8 and 9 a. m. For this investigation, however, most of the analyses were made on samples taken at weekly intervals, although, as previously mentioned, samples were taken more frequently at certain stages in the development of the plants. The plants were taken up by the roots, freed of soil, and air-dried. The roots were cut off, and later, during heading time, the heads were also cut off, and both were discarded. The stalks and leaves were ground fine enough to pass through a 60-mesh sieve, and dried in an oven at 100° C. For the determination of the percentages of alcohol-benzene, cold and hot water, and 1 percent of hydrochloric acid extractives, as well as for the determination of lignin, plant material ground to pass through an 80-mesh sieve was used. The following determinations were made, oven-dried plant material being used for each.

Ash.—This was determined in the usual manner by igniting a weighed sample with a Bunsen burner and weighing the inorganic residue.

Nitrogen.—All nitrogen determinations were made in the usual manner by the Kjeldahl-Gunning-Arnold method (*1*).

Methoxyl in original plant material.—The percentage methoxyl in the dried unextracted plant material was determined as described by one of the writers in a previous publication (*26*). The methyl iodide was absorbed in pyridine.

Methoxyl in extracted plant material.—The plant material was successively extracted with a 1 : 2 alcohol-benzene solution, cold water, hot water, and a 1-percent hydrochloric acid solution, and the percentage loss in weight due to these extractions was determined. This operation removed fatty and waxy substances, water-soluble carbohydrates, and proteins, and it also removed any methoxyl groups occurring as methyl esters of organic acids, such as, for example, is found in the pectins. The percentage of methoxyl in the plant material left after extraction was then determined by the method referred to above, and the result was calculated on the basis of the original unextracted material. The percentage of methoxyl thus obtained represents essentially lignin methoxyl, together with some firmly bound methoxyl occurring in some other components of the plant.

Alcohol-benzene extractives.—These were determined by extracting a weighted sample in a Soxhlet extractor for 30 hours with a 1 : 2 alcohol-benzene solution, and ascertaining the loss of weight.

Cold-water extractives.—To a weighed sample of the dry material which had been extracted with alcohol-benzene solution as described above, distilled water was added in the proportion of 150 cc to 1 g of sample and allowed to digest at room temperature with frequent stirring for 48 hours. The loss in weight after extracting and redrying was calculated on the basis of the original unextracted material.

Hot-water extractives.—A weighed sample of the dry residue from the previous extraction was treated with distilled water in the proportion of 150 cc of water to 1 g of sample and boiled under a reflux condenser for 3 hours. The loss in weight was calculated on the basis of the original dry unextracted material.

One-percent hydrochloric acid extractives.—A weighed sample of the plant material which had been successively extracted with alcohol-benzene solution, cold water, and hot water, as described above, was

treated with a 1-percent hydrochloric acid solution in the proportion of 150 cc of acid solution to 1 g of plant material and boiled under a reflux condenser for 3 hours. The loss in weight was calculated on the basis of the original dry unextracted material.

Total extractives.—This represents the sum of the percentages of the alcohol-benzene, cold-water, hot-water, and 1-percent hydrochloric acid extractives.

Uronic acid anhydrides.—These were determined in the dry unextracted material according to the procedure recommended by Dickson, Otterson, and Link (*6*), as modified slightly by Phillips, Goss, and Browne (*28*). The uronic acids are present in the pectins and in the hemicelluloses.

Total furfural.—This was determined in the dry unextracted plant material by the method of the Association of Official Agricultural Chemists (*1*).

Pentosans.—From the percentage of total furfural determined as described above, one-sixth of the percentage of uronic acid anhydrides, determined by the method referred to above, was deducted, and the result was calculated as percentage pentosans. The figure thus obtained represents the sum of the furfural yielded by the pentoses of the polyuronides,[4] plus the furfural of the Cross and Bevan cellulose, all calculated as percentage of pentosans.

Cross and Bevan cellulose.—This was determined by the method described by one of the writers in a previous publication (*26*). The percentage of ash in the Cross and Bevan cellulose was determined, and the result was calculated on the basis of ash-free material.

Furfural in Cross and Bevan cellulose.—This was determined by the method of the Association of Official Agricultural Chemists (*1*). The percentage of furfural was calculated on the basis of ash-free Cross and Bevan cellulose.

Cellulose.—The Cross and Bevan cellulose consists essentially of two components, a "true" cellulose fraction and a furfural-yielding fraction, which in all probability is xylan (*20*). In order to ascertain the "true" cellulose content of the material, the percentage of furfural in the Cross and Bevan cellulose determined by the method referred to above was calculated as percentage of pentosans, and when this was deducted from the percentage of Cross and Bevan cellulose, the result was the percentage of "true" cellulose in the sample.

Lignin.—The lignin determinations were made on plant material which had been extracted with alcohol-benzene solution, cold water, hot water, and a 1-percent hydrochloric acid solution, according to the procedure described above. The lignin was determined by the fuming hydrochloric acid method described by one of the writers in a previous publication (*26*). Three samples were weighed, and in the lignin residues from the first two samples the percentages of ash and nitrogen, respectively, were determined; in the lignin residue from the third sample the percentage of methoxyl was determined. Corrections were made for the nitrogen and ash in the lignin.

Methoxyl in ash-free lignin.—The percentage of methoxyl in the lignin residue was determined by the method referred to above, and the result was calculated on the basis of ash-free lignin.

[4] The term "polyuronides" was introduced into chemical literature by Candlin and Schryver (*3*). It refers to a group of substances, widely distributed in the plant kingdom, which are formed by the conjugation of certain sugar acids (glucuronic and galacturonic acids) with sugars. The hemicelluloses and the pectins are, therefore, polyuronides.

TABLE 1.—*Percentage composition of barley plants at different stages of growth*

[Results calculated on the basis of oven-dried material. Figures in parentheses give percentages on the basis of ash-free material]

Age of plants (days)	Ash in original material	Nitrogen in original plant material	Methoxyl in original material	Methoxyl in extracted plant material [1]	Alcohol-benzene extractives	Cold water extractives	Hot water extractives	1 per cent hydrochloric acid extractives	Total extractives	Uronic acids (as anhydrides)	Total furfural	Pentosans [2]	Cross and Bevan cellulose	Furfural in Cross and Bevan cellulose [3]	Cellulose	Lignin	Nitrogen in crude lignin	Methoxyl in ash-free lignin	Ash in lignin	Methoxyl in "pure" lignin
7 [4]	13.68	----------	0.54 (.62)	0.14 (.16)	24.83 (28.76)	17.82 (20.64)	5.74 (6.65)	29.58 (34.26)	77.97 (90.31)	6.16 (7.13)	6.31 (7.31)	9.04 (10.47)	----------	----------	----------	1.46 (1.71)	6.95 (8.87)	----------	21.61	----------
14 [5]	13.61	5.87 (6.79)	.58 (.67)	.14 (.16)	30.48 (35.28)	17.28 (20.00)	4.60 (5.43)	27.28 (31.58)	79.73 (92.29)	5.88 (6.80)	5.86 (6.78)	8.34 (9.65)	22.6 (27.0)	9.36	19.0 (22.7)	1.71 (1.98)	6.76 (8.62)	2.03	21.61	4.42
21	16.48	6.08 (7.28)	.59 (.70)	.15 (.18)	27.70 (33.16)	16.18 (19.37)	4.15 (4.96)	28.34 (33.93)	76.37 (91.42)	6.60 (7.90)	6.66 (7.97)	9.33 (11.17)	26.0 (31.1)	13.20	20.1 (24.0)	2.31 (2.76)	5.49 (7.00)	2.76	21.61	4.90
29	15.83	5.06 (6.01)	.63 (.74)	.16 (.19)	27.55 (32.73)	17.55 (20.84)	4.77 (5.66)	23.80 (28.27)	72.27 (85.84)	6.52 (9.29)	7.82 (9.29)	11.14 (13.23)	28.0 (30.9)	13.20	21.9 (23.9)	2.50 (2.97)	3.37 (5.76)	3.93	41.47	6.12
35	14.20	3.86 (4.49)	.83 (.96)	.31 (.36)	14.84 (17.29)	30.01 (34.98)	4.85 (5.65)	20.56 (23.96)	70.26 (81.88)	6.78 (6.75)	7.71 (8.99)	11.53 (13.43)	28.9 (33.7)	14.19	21.9 (25.5)	2.88 (3.35)	3.37 (5.28)	4.88	36.23	7.27
42	12.35	3.08 (3.51)	1.17 (1.33)	.64 (.73)	17.23 (19.68)	17.69 (20.18)	5.75 (6.56)	26.20 (29.89)	66.89 (76.31)	6.26 (6.00)	7.92 (9.03)	12.04 (13.72)	35.4 (40.4)	16.19	20.9 (23.9)	3.49 (3.98)	3.69 (4.99)	6.55	25.98	9.51
49	10.12	1.95 (2.17)	1.77 (1.97)	1.06 (1.18)	13.21 (14.69)	14.27 (15.87)	6.39 (7.11)	27.37 (30.45)	61.24 (68.12)	6.90 (6.45)	8.29 (9.22)	12.78 (14.22)	39.0 (43.4)	15.66	28.2 (31.6)	5.10 (5.67)	3.20 (3.76)	9.91	14.91	12.94
56	10.29	1.77 (1.97)	2.00 (2.23)	1.22 (1.36)	14.41 (16.06)	13.18 (14.69)	5.90 (6.57)	25.68 (28.62)	59.17 (65.94)	4.76 (5.30)	12.64 (14.09)	20.25 (22.57)	36.8 (41.0)	14.33	27.8 (31.0)	5.93 (6.61)	3.09 (3.70)	10.89	16.40	14.17
63	8.66	1.59 (1.74)	2.23 (2.44)	1.39 (1.52)	17.98 (19.68)	10.48 (11.47)	4.93 (5.39)	25.29 (27.68)	58.68 (64.22)	4.90 (5.36)	12.06 (13.20)	19.22 (21.04)	37.4 (40.9)	13.68	28.7 (31.4)	6.80 (7.44)	3.18 (3.50)	11.62	9.22	14.71
70	9.40	1.52 (1.67)	2.45 (2.70)	1.56 (1.72)	12.89 (14.33)	9.50 (10.48)	4.67 (5.15)	26.44 (29.18)	53.60 (59.14)	5.92 (6.53)	14.16 (14.96)	21.49 (23.71)	43.7 (48.2)	15.43	32.2 (35.5)	6.83 (3.25)	3.25 (3.55)	12.62	8.81	16.24
77	7.77	.98 (1.06)	2.38 (2.58)	1.42 (1.54)	17.94 (19.45)	9.63 (10.44)	4.55 (4.93)	26.34 (28.55)	58.46 (63.37)	4.96 (5.37)	14.16 (15.35)	22.79 (24.71)	43.1 (46.7)	17.45	30.3 (32.8)	6.97 (7.55)	1.61 (1.86)	14.10	13.64	15.94
84	8.22	.71 (.77)	2.64 (2.76)	1.46 (1.59)	14.52 (15.82)	13.31 (14.50)	4.66 (5.07)	26.20 (28.54)	58.69 (63.93)	4.30 (4.68)	13.95 (15.19)	22.62 (24.64)	40.8 (44.4)	14.17	30.9 (33.7)	7.34 (7.99)	1.24 (1.46)	14.72	15.35	16.20
86	7.49	.59 (.63)	2.70 (2.91)	1.69 (1.82)	13.54 (14.65)	12.76 (13.79)	2.83 (3.05)	27.15 (29.34)	56.28 (60.81)	4.42 (4.78)	14.70 (15.99)	23.87 (25.80)	41.4 (44.7)	13.84	31.6 (34.1)	7.74 (8.36)	1.09 (1.12)	15.18	11.02	16.31

[1] Results calculated on the original unextracted plant material.
[2] Corrected for furfural from uronic acids.
[3] Ash-free basis.
[4] This sample was a composite of plants that were 1, 2, 3, 4, 5, 6, and 7 days old, respectively.
[5] This sample was a composite of plants that were 8, 9, 10, 11, 12, 13, and 14 days old, respectively.

Methoxyl in pure lignin.—The percentage of methoxyl in the lignin residue was determined, and the result was calculated on the basis of ash-free and crude protein-free (N×6.25) lignin. It is necessary to emphasize in this connection that the term "pure lignin" is used merely to designate the lignin residue from which proper deductions were made for ash and crude protein, and should not be confused with the term as applied to crystalline organic compounds.

RESULTS

The results obtained are given in 3 tables and illustrated graphically in 6 figures. Table 1 shows the percentage composition of barley plants at different stages of growth.

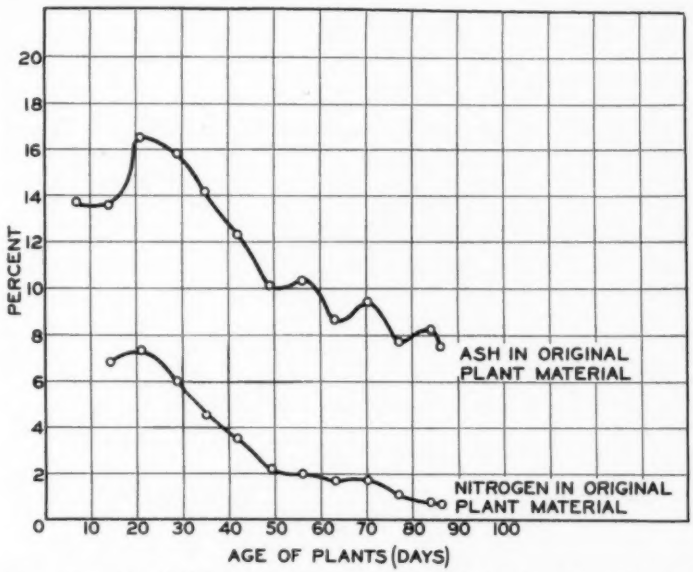

FIGURE 1.—Percentages of ash and nitrogen in leaves and stalks of barley plants at successive stages of growth. (The nitrogen values are on ash-free basis.)

ASH

After an initial rise in the percentage of ash, which reached a maximum of 16.48 percent in the third sampling period, there was a progressive although somewhat irregular decrease as the plants grew older (fig. 1). This is in agreement with the results obtained by Lawes and Gilbert (*17*). Results of a similar nature were obtained with wheat plants by Phillips, Davidson, and Weihe (*27*), and by Malhotra (*18, 19*). Norman (*21*), working with barley plants, also obtained results of a similar character.

NITROGEN

The percentage of nitrogen also showed an increase in the early stages of growth, after which there was a steady decrease (fig. 1). The data obtained by Shaw and Wright (*32*) in their studies on the

percentage of nitrogen in the corn plant at different stages of growth showed a similar tendency. Phillips, Davidson, and Weihe (*27*) found a similar decline in the percentage of nitrogen in plants at different stages of growth.

METHOXYL IN ORIGINAL PLANT MATERIAL

In determining the percentage of methoxyl in a plant material by the Zeisel method (*26*), one obtains not only the percentage of methoxyl found in methyl ethers, but also that present in esterlike combination, as in pectins and in the methyl esters of organic acids generally. The figures listed under this heading represent, therefore, the total methoxyl content of the plant material, that is to say, the

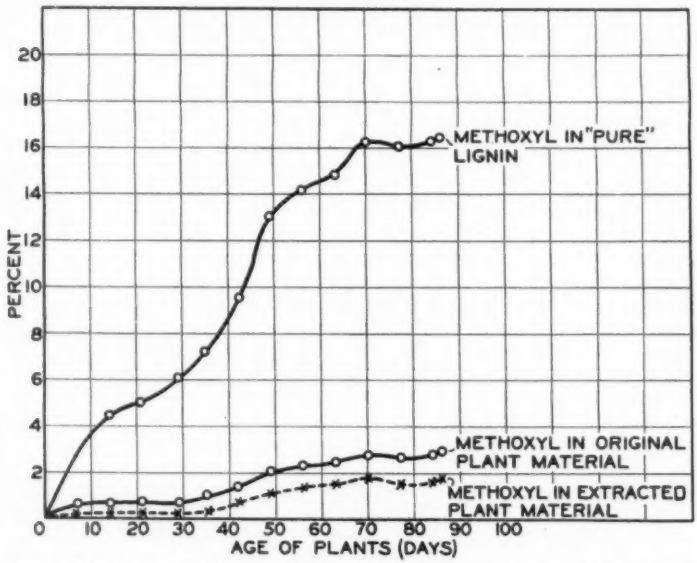

FIGURE 2.—Percentages of methoxyl in original plant material, methoxyl in extracted plant material, and methoxyl in "pure" lignin (all on ash-free basis) in leaves and stalks of barley plants at successive stages of growth.

sum of the percentage of methoxyl present either in the form of methyl ethers or methyl esters. In the early stages of growth the percentage of methoxyl in the plant material was fairly constant (fig. 2). Subsequently there was a progressive rise, presumably caused by the process of lignification, which is accelerated as the plant grows older. The results obtained by Phillips, Davidson, and Weihe (*27*) with wheat plants show a similar trend, although the percentages of methoxyl in the culms of the mature wheat plants were higher than those recorded in table 1.

METHOXYL IN EXTRACTED PLANT MATERIAL

The percentages of methoxyl in extracted plant material recorded in table 1 represent essentially methoxyl groups present as methyl ethers. The percentages of methoxyl recorded here represent es-

sentially lignin methoxyl groups, although not entirely, as will be pointed out later. It will be noted that in this case, as in the methoxyl in original plant material, the percentage of methoxyl increases with the age of the plant (fig. 2). It will be noted also that the percentage of lignin likewise increases as the plant grows older, so that, on the supposition that the methoxyl in the extracted plate material represents essentially lignin methoxyl, the results are what one would expect.

ALCOHOL-BENZENE EXTRACTIVES

The alcohol-benzene extractives include a heterogeneous class of compounds, as the solvent employed removes various resinous, fatty, or waxy substances. The results show that there was a decrease in the percentage of alcohol-benzene extractives as the plants grew older. From a percentage of more than 30 in the second sampling period, it decreased to 13.54 percent in the last sampling period. The decrease, however, was neither even nor regular.

COLD- AND HOT-WATER EXTRACTIVES

After a slight decrease in the second and third sampling periods, the percentage of cold-water extractives increased, reaching a maximum in the fifth sampling period. The percentage then declined rapidly, although not in a regular manner. The percentage of hot-water extractives at first declined from 6.65 (calculated on ash-free material) to 4.96, then it increased until it reached a maximum of 7.11 in the seventh sampling period. Subsequently, however, it declined again, so that in the last sampling period the percentage amounted to only 3.05.

1-PERCENT HYDROCHLORIC-ACID EXTRACTIVES

The data on the 1-percent hydrochloric-acid extractives are irregular, partly owing to the heterogenous nature of the extract, as the treatment with acid hydrolyzes some of the hemicelluloses, the polyuronides, the pentosan fraction of the Cross and Bevan cellulose, and perhaps some of the proteins.

TOTAL EXTRACTIVES

The figures presented under this heading represent the sum of the percentages of alcohol-benzene, cold water, hot water, and 1-percent hydrochloric acid extractives. The percentage of total extractives decreased as the plant grew older. There was a slight increase in the second sample, as compared with the first. Subsequently, there was a gradual decrease in the percentage. Attention is called to the fact that, particularly in the early stages of the development of the plant, approximately 80 percent of the plant substance can be removed by successive extraction with 1:2 alcohol-benzene solution, cold water, hot water, and hot 1-percent hydrochloric acid.

URONIC ACIDS

The percentage of uronic acids (calculated as anhydrides) increased during the early development of the plant and reached a maximum when the plants were 29 days old (fig. 3). After that there was a gradual, though not altogether regular, decrease in the percentage of uronic acids. These acids are found chiefly in two structural com-

ponents of the plant, namely, the pectins and the hemicelluloses. As the plants grow older, the pectin content, particularly of lignified tissues, decreases, so that the uronic acids in the plant would naturally show a decrease. Moreover, the pectins contain a much greater percentage of uronic acids than do the hemicelluloses, so that the decrease in pectin would account for a much greater loss in percentage of uronic acids than an equal loss in the hemicelluloses. Attention is called again to the observation of Candlin and Schryver (*3, p. 376*):

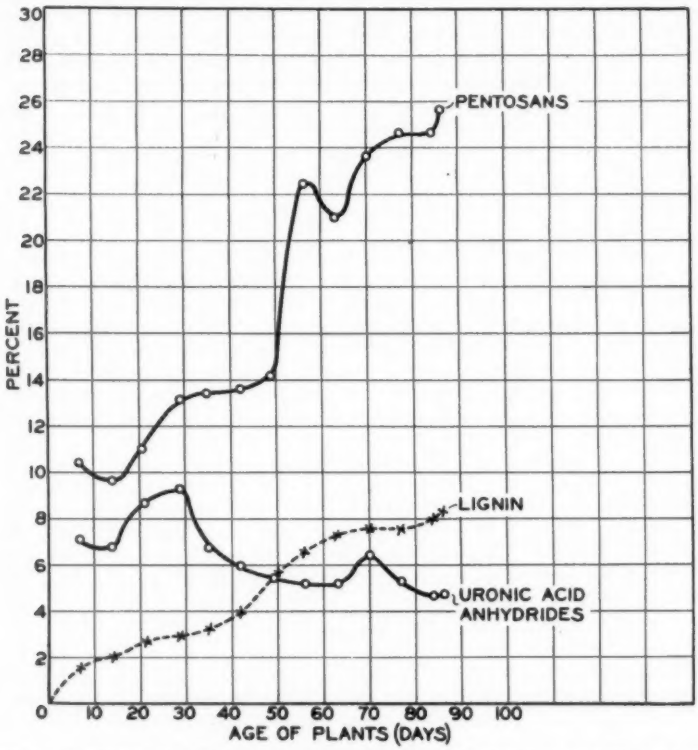

FIGURE 3.—Percentages of uronic-acid anhydrides, pentosans, and lignin in leaves and stalks of barley plants at successive stages of growth.

Lignified tissues contain lignins and hemicelluloses in relatively large amounts, with only traces (if any) of pectins. Non-lignified tissues, on the other hand, contain relatively large amounts of pectins, small amounts of hemicelluloses, and no lignin.

TOTAL FURFURAL AND PENTOSANS

The furfural obtained when a plant material is distilled with 12-percent hydrochloric acid is derived from two groups, namely, the pentoses or pentosans, and the uronic acids. These two groups are found in the gums, hemicelluloses, and pectins. A pentose or pentosan unit is also associated with the cellulose in the Cross and Bevan cellulose. The figures on the total furfural in the leaves and stalks

of the barley plants represent, therefore, the sum of the percentages of furfural yielded by the uronic acids and the pentose or pentosan units. The percentage of furfural increased with the increase in the age of the plant.

The figures on the percentage of pentosans were obtained by deducting from the percentage of total furfural the percentage of furfural arising from the uronic acids (the uronic acids yield one-sixth of their weight of furfural) and calculating the difference as pentosans. These figures represent, therefore, the total pentose or pentosan units in the plant material, irrespective of whether these units occur in the gums, hemicelluloses, pectins, or in Cross and Bevan cellulose. Table 1 and figure 3 show that while in the second sampling period

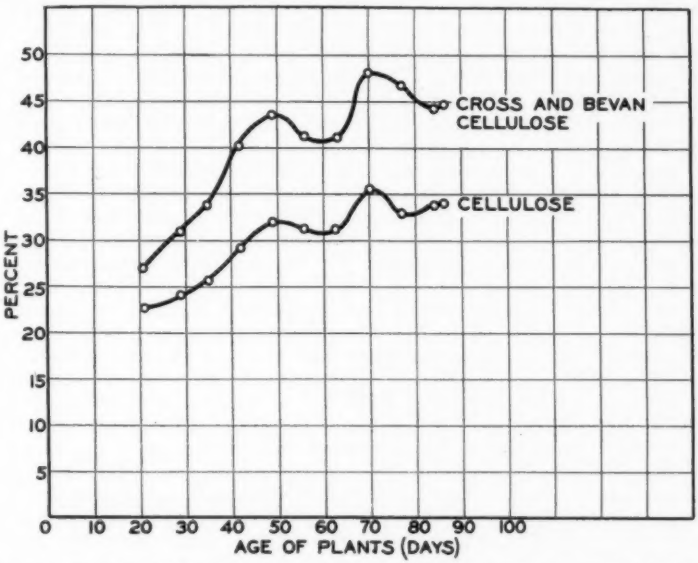

FIGURE 4.—Percentages of Cross and Bevan cellulose and cellulose in leaves and stalks of barley plants at successive stages of growth.

there was a small decrease in the percentage of pentosans as compared with the material from the first sampling period, the plants from the subsequent samplings, with one exception, showed a steady increase until maturity, when the highest percentage was obtained.

CROSS AND BEVAN CELLULOSE, FURFURAL IN CROSS AND BEVAN CELLULOSE, AND CELLULOSE

The percentage of Cross and Bevan cellulose, as well as the percentage of cellulose, increased rapidly as the plant grew older (fig. 4). The maximum percentage was reached when the plants were 70 days old. After this, as the plants matured, the percentages of Cross and Bevan cellulose, as well as cellulose, decreased slightly. When taken in conjunction with the data on the lignin content at various stages of growth of the plant, these data are interesting. As both the

percentage of cellulose and the percentage of lignin increased as the plants grew older, there is no direct evidence that the lignin increased at the expense of the cellulose.

The percentages of furfural in the Cross and Bevan cellulose increased as the plants grew older. The increase, however, was neither consistent nor even. The maximum percentage was reached when the plants were 77 days old, after which there was a decrease, and at maturity the percentage amounted to 13.84, as compared with 17.45 when the plants were 77 days old.

LIGNIN, NITROGEN, ASH, AND METHOXYL IN LIGNIN

Table 1 and figure 3 show that the lignin increased consistently as the plants grew older. In the first sample the percentage of lignin, calculated on the organic portion of the plant material, amounted to 1.71, and in the last sample, when the plants were mature, the percentage of lignin was 8.36. The lignin values obtained in this investigation are quite different from those recorded by Norman (*21*), although the tendency was the same. According to the results of this investigator, the young seedlings contained more than 14 percent of lignin. The percentage of lignin then increased until a month before maturity, when it amounted to 19.7, after which there was a slight fall. The decided difference between the lignin values recorded in this paper and those reported by Norman is due to the fact that different methods were employed for the quantitative estimation of lignin, Norman using the old method of Ost and Wilkening (*24*). While none of the methods now employed for the quantitative estimation of lignin is entirely free from criticism, the method of Ost and Wilkening is particularly objectionable, as the residue obtained when the plant material is hydrolyzed with 72 percent sulphuric acid is assumed to be lignin. This is now known to be incorrect, as certain fatty or waxy substances and their degradation products are not removed by the treatment with 72 percent sulphuric acid, and are weighed along with the lignin. Moreover, as was shown by Paloheimo (*25*), and recently confirmed by Hilpert and Littmann (*13*), certain carbohydrates when treated with 72 percent sulphuric acid yield insoluble huminlike products. These complexes, therefore, would also be weighed along with the lignin. Furthermore, according to the method of Ost and Wilkening, no correction is applied for the nitrogenous complexes with which the lignin residue is nearly always contaminated. While the percentage of nitrogen in the lignin residue of wood is generally small, this is not the case in the lignin residue from young plants. Attention is called to the nitrogen values in the crude lignin recorded in table 1 and shown graphically in figure 5. The percentage of nitrogen in the lignin residue from the first sample amounted to nearly 7. As the plant grew older, the percentage of nitrogen in the lignin residue decreased, until in the lignin from the mature plant it amounted to only 1. No doubt this was due to the general diminution in the nitrogen content of the leaves and stalks

as the plant grew older. (See figures in table 1 on percentage of nitrogen in original plant material.) The high lignin values obtained when the Ost and Wilkening method is used for the determination of lignin in such materials as young and mature barley plants undoubtedly are due to a contamination of the crude lignin residue with nitrogenous complexes, with huminlike products derived from certain carbohydrates, and with fatty or waxy products and their degradation products. Norman recognized the unsatisfactory character of the method he employed for the determination of lignin, and he states that "the figures are presented with some reserve."

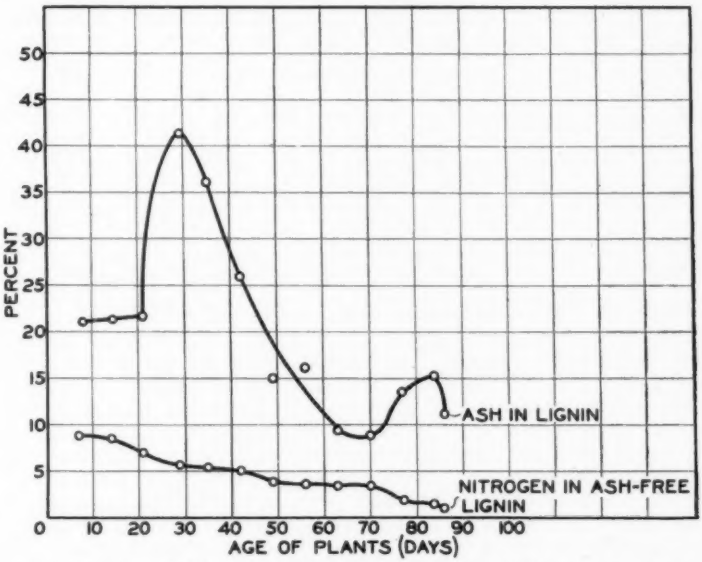

FIGURE 5.—Percentages of ash in lignin and nitrogen in ash-free lignin isolated from leaves and stalks of barley plants at successive stages of growth.

The figures on the percentages of methoxyl in the ash-free lignin and in the so-called "pure" lignin (fig. 2) indicate a decided difference in the composition of lignin, at least so far as its degree of methylation is concerned, isolated from plants at different stages of development. As the plant grows older, not only does the percentage of lignin increase, but there is also a steady increase in the percentage of methoxyl in the lignin.

The percentage of ash in the lignin varied considerably. After an initial increase it decreased, but not consistently (fig. 5).

TABLE 2.—*Percentage of furfural yielded by several components of barley plants at successive stages of growth*

[Results calculated on basis of ash-free plant material]

Age of plants (days)	Total furfural	Furfural from uronic acids		Furfural from pentose of Cross and Bevan cellulose		Furfural from pentoses of polyuronides [1]		Furfural from pentoses of polyuronides calculated as xylose
	Percent	*Percent*	*Percent of total*	*Percent*	*Percent of total*	*Percent*	*Percent of total*	*Percent*
7 [2]	7.31	1.19	16.2					
14 [3]	6.78	1.13	16.6					
21	7.97	1.44	18.0	2.52	31.6	4.01	50.4	6.96
29	9.29	1.54	16.5	4.08	43.9	3.67	39.6	6.37
35	8.99	1.12	12.5	4.78	53.2	3.09	34.3	5.33
42	9.03	1.00	11.0	6.54	72.4	1.49	16.6	2.58
49	9.22	.91	9.7	6.80	73.7	1.51	16.4	2.62
56	14.09	.88	6.2	5.87	41.6	7.34	52.1	12.74
63	13.20	.89	6.8	5.59	42.7	6.72	50.5	11.47
70	14.96	1.09	7.2	7.46	49.8	6.41	43.0	11.12
77	15.35	.89	5.8	8.15	53.0	6.31	41.2	10.95
84	15.19	.78	5.1	6.29	41.4	8.12	53.5	14.09
86	15.99	.79	4.9	6.19	38.7	9.01	56.4	15.64

[1] By difference.
[2] This sample was a composite of plants that were 1, 2, 3, 4, 5, 6, and 7 days old, respectively.
[3] This sample was a composite of plants that were 8, 9, 10, 11, 12, 13, and 14 days old, respectively.

Table 2 shows the percentages of furfural yielded by several components of the leaves and stalks of the barley plants at successive stages of growth (fig. 6). Instead of calculating the percentage of total furfural as pentosans in the conventional manner, an attempt was made to separate the percentages of furfural yielded by the several components of the plant material, thus affording a more accurate picture of the development of the furfural-yielding components. The figures in the second column are taken from Table 1 and are inserted here for the sake of completeness and comparison. The figures in the third column were obtained by dividing the percentages of uronic anhydrides by 6 (the uronic acid anhydrides yield one-sixth of their weight of furfural). The figures in the fifth column were obtained by multiplying in each case the percentage of Cross and Bevan cellulose by the percentage of furfural in the Cross and Bevan cellulose, and dividing the result by 100. The figures in the seventh column were obtained in each case by subtracting the sum of the percentages recorded in the third and fifth columns from the percentage of total furfural given in the second column. The figures given in the last column were calculated from those recorded in the seventh column, taking into consideration the fact that xylose yields only 90 percent of the theoretical quantity of furfural. Table 2 and figure 6 show that after a slight initial increase the percentage of furfural yielded by the uronic acids tended to decrease as the plants matured. The percentage of furfural yielded by the Cross and Bevan cellulose showed an upward trend, with a small decrease in the last two sampling periods. In general, the results indicated that the percentage of furfural-yielding fraction of the Cross and Bevan cellulose increased as the plants matured, and in the later stage of the development (77 days), it furnished more than 50 percent of the total furfural. The percentage of furfural due to the pentoses of the polyuronides showed a decrease in the first few weeks of the development of the

plants, and then it suddenly increased to such an extent that at maturity 56 percent of the total furfural was furnished by the pentoses of the polyuronides. The figures in the last column, of course, show the same tendency as those recorded in the seventh column.

Data are presented in table 3 on the distribution of the firmly bound methoxyl groups between the lignin and the nonlignin constituents of the barley plants at successive stages of growth. The table shows that the weight of lignin methoxyl in 100 g of plant material increased consistently as the plant matured, but the percentage of lignin methoxyl calculated on the basis of the total firmly bound methoyxl did not increase in a regular fashion, although the figures show an upward trend. In the mature plants, from 75 to 80 percent of the total firmly bound methoxyl is found in the lignin.

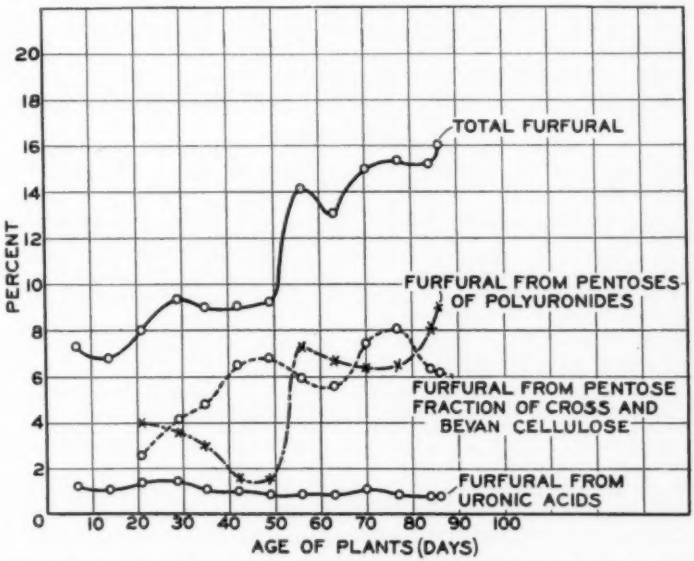

FIGURE 6.—Percentages of furfural yielded by several components of leaves and stalks of barley plants at successive stages of growth.

The percentage of methoxyl not in the lignin decreased, in the main, as the plant matured. In the early stages of the development of the plant, the percentage of methoxyl not in the lignin seemed rather high. It has been shown by O'Dwyer (*23*), Schmidt and his coworkers (*30*), and Hägglund and Sandelin (*12*) that in wood some firmly bound methoxyl groups are found also in the carbohydrate fraction, although the percentages recorded by these investigators are lower. Whether the firmly bound methoxyl obtained from the nonlignin constituents of the plant material represents real methoxyl groups or methyl groups attached directly to carbon which have been split out by the hydriodic acid employed in the determination of methoxyl is not known. The nature of the substance or substances yielding the firmly bound methoxyl not associated with the lignin merits further investigation.

TABLE 3.—*Distribution of firmly bound methoxyl between lignin and nonlignin constituents of barley plants at successive stages of growth*

[Results calculated on basis of ash-free plant material]

Age of plants (days)	Firmly bound methoxyl in 100 g of plant material	Lignin methoxyl in 100 g of plant material	Lignin methoxyl	Methoxyl not in lignin
	Grams	*Grams*	*Percent of total*	*Percent of total*
7 [1]	0.16			
14 [2]	.16	0.09	56.2	43.8
21	.18	.13	72.2	27.8
29	.25	.18	72.0	28.0
35	.36	.24	66.6	33.4
42	.73	.38	52.0	48.0
49	1.18	.73	61.8	38.2
56	1.36	.94	69.1	30.9
63	1.52	1.09	71.7	28.0
70	1.72	1.24	72.0	28.1
77	1.54	1.20	77.9	22.9
84	1.59	1.29	81.1	18.3
86	1.82	1.36	74.7	25.3

[1] This sample was a composite of plants that were 1, 2, 3, 4, 5, 6, and 7 days old, respectively.
[2] This sample was a composite of plants that were 8, 9, 10, 11, 12, 13, and 14 days old, respectively.

DISCUSSION

Cross and Bevan (*4*) advance the following theory of lignification (*4, pp. 180–181*):

* * * the process of lignification consists in a series of progressive and intrinsic modifications of a cellulose or oxycellulose tissue, the products of modification remaining associated with the residues of the parent substance in a state of combination or of intimate mixture, the final products of metabolism (aromatic products, pentosans, &c.) being excreted and taking no further part in the organic processes of the tissue.

Among others, they advance the following argument in favor of their theory of lignification (*4, p. 179*):

Regarding lignification as a process of continuous modification of cellulose, and the woods as representing the extreme limits of such a process, these should show an increase in lignone at the expense of cellulose; which is in fact the case. Lignocelluloses in the first year of growth contain 70–80 p. ct. cellulose; the woods, on the other hand, 50–60 p. ct.

While it is true that woods generally contain a greater percentage of lignin than is found in annual plants, it is by no means certain that the evidence offered by Cross and Bevan is an indication that the plant synthesizes lignin from cellulose. The results of this investigation show that the period in which lignin increased was also that in which a considerable increase was obtained in the cellulose. Both the lignin and the cellulose increased progressively with the age of the plant. No direct evidence was obtained that the lignin increased at the expense of the cellulose. If the Cross and Bevan conception is correct, in consideration of the results obtained in this investigation, the assumption would have to be made that the plant synthesizes cellulose faster than it converts it into lignin.

With reference to the hypothesis of Rassow and Zschenderlein (*29*) that lignin is formed by the plant from pentoses or pentosans, the results obtained in this investigation fail to show any direct relationship between the content of pentose material and lignin. Both the percentage of lignin and the percentage of pentoses increased as the plant matured, and here also, if the hypothesis of Rassow and Zschenderlein is correct, it would be necessary to assume that the plant synthesizes pentoses faster than it converts them into lignin.

The results obtained in this investigation reveal that the percentages of uronic acids and soluble sugars (cold- and hot-water extractives) decreased as the plant matured. In view of the fact that the total pentose material increased with the age of the plant and that soluble pentoses are also included among the cold- and hot-water extractives, the loss of soluble carbohydrates other than pentoses must have been even greater than the figures on the cold- and hot-water extractives reveal. It is, of course, recognized that much of this loss was due to a translocation of the hexoses to the seed where it was stored in the form of starch; it is nevertheless conceivable that part of the hexose sugars may have been utilized by the plant in the production of lignin. Whether the synthesis of lignin proceeds directly or whether the hexoses are first oxidized to uronic acids is, of course, not known. While the results obtained in this investigation do not disprove the hypotheses of Cross and Bevan and of Rassow and Zschenderlein, they definitely do not support them. In the main, the results are more in harmony with Schrauth's conception as to the genesis of lignin in the plant.

SUMMARY

A study was made of the composition of the leaves and stalks of the barley plant at successive stages of growth.

After an initial increase, the percentages of ash and nitrogen declined steadily as the plant matured.

The percentage of methoxyl in the original and in the extracted plant materials increased with the age of the plant.

The percentages of alcohol-benzene, cold-water, and hot-water extractives declined, though not in a regular manner, as the plant became older. The 1-percent hydrochloric acid extractives showed no definite tendency.

The percentage of uronic acids increased somewhat during the early development of the plant, and then declined as the plant matured. The percentage of the furfural-yielding components, as well as the percentage of pentoses calculated as pentosans, increased as the plant matured. After a slight initial increase, the percentage of furfural yielded by the uronic acids tended to decrease, and in no case did it amount to more than 18 percent of the total. The percentage of furfural furnished by the Cross and Bevan cellulose showed an upward trend at first, but decreased somewhat as the plants matured. The furfural derived from the pentoses of the polyuronides showed a decrease in the first few weeks of the development of the plants, and then suddenly increased to such an extent that at maturity 56 percent of the total furfural was furnished by the pentoses of the polyuronides.

The percentages of Cross and Bevan cellulose, as well as the cellulose, increased rapidly as the plants grew older. As the plants matured there was a slight decrease in the percentage of these two components.

The percentage of lignin, as well as the methoxyl in the lignin, increased in a regular manner as the plants developed and matured. The lignin from young plants differs from the lignin of mature plants in that the former contains a much smaller percentage of methoxyl. As the plants mature not only does the percentage of lignin greatly

increase, but there is also a rapid methylation of the hydroxyl groups of the lignin.

In the mature plants, 75 to 80 percent of the firmly bound methoxyl groups are found in the lignin.

No direct evidence was obtained that the barley plant synthesizes lignin from cellulose, pentoses, or pentosans. The results obtained are more in harmony with the hypothesis that the barley plant synthesizes lignin from soluble sugars other than pentoses.

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THE DISTRIBUTION AND CONDITION OF PHOSPHORUS IN THREE HORIZONS OF A DIFFERENTIALLY FERTILIZED HAGERSTOWN CLAY LOAM SOIL PLANTED TO APPLE TREES IN METAL CYLINDERS [1]

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INTRODUCTION

The writer has recently reported (*47*)[2] the distribution and condition of the nitrogen in the soil during an experiment with apple trees grown in metal cylinders for a period of 6½ years, and which received annually for the last 3 years of growth different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate. The distribution and condition of the phosphorus is reported in the present paper. The principal objective was to determine the condition of the residual phosphoric acid (P_2O_5) derived from the added monocalcium phosphate. 98.7 and 87.3 percent of the phosphoric acid was still present in the surface 0 to 7 inches of the NPK-treated cylinders under sod and cultivation, respectively, at the end of the experiment.

The mechanism of the absorption by soils of certain anions (phosphate, tartrate, oxalate, and citrate) is comparable in certain respects to that of cations, that is, it is one of exchange involving equilibrium (*8*, *9*, *10*). The characteristics of these exchange reactions are that they take place in the boundary between two phases, and that consequently no distinction can be made between absorption, adsorption, and chemical reaction in this interface. All such reactions may be described as disperse reactions (*50*).

The physicochemical examination of the system phospate, ferric hydroxide, aluminum hydroxide, calcium (and magnesium) hydroxide by Gaarder (*21*) has thrown considerable light on the course of the reactions that might occur under soil conditions, albeit the experiments were in vitro, i. e., they were not conducted with soils present. These experiments of Gaarder show that the systems resulting depend on the concentrations of hydrogen, iron, aluminum, calcium, and magnesium ions relative to the phosphate ions. When the conditions are such that the concentrations of iron, aluminum, and calcium are in excess of that of the phosphate only two narrow pH ranges were observed at which the concentrations of phosphate (PO_4) were more than 0.01 mg per liter, viz, at 3.9 to 4.5 and 6.7 to 7.3.

The approach to the problem of the condition of the residual phosphate applied in the writer's cylinder experiments has been made by ascertaining the total amount of phosphorus present in the three horizons, 0 to 7, 7 to 21, and 21 to 53 inches, respectively, of the soils of the treated and untreated cylinders, together with the determination of the amounts extracted from these soils under specified conditions by solvents having a wide range of pH values.

[1] Received for publication Mar. 18, 1935; issued October 1935. Pennsylvania Agricultural Experiment Station, Technical Paper no. 683.

[2] Reference is made by number (italic) to Literature Cited, p. 336.

METHODS OF EXPERIMENTATION

FIELD PLAN OF THE CYLINDER EXPERIMENTS

The detailed plan of this experiment has appeared in a number of publications (*2, 45, 46*); only a brief outline, therefore, is necessary.

The soil used in the cylinders is a virgin soil of Trenton formation formed by the weathering of limestone (*44*). In the present paper this soil is designated the "original soil." The excavation was made on a strip of land 110 feet by 11 feet near the college experimental orchard. The surface horizon is a heavy silt loam and is underlain by a clay loam which becomes heavier in texture with depth (*44*). During the excavation the three horizons, viz, surface (0 to 7 inches), subsurface (7 to 21 inches), and subsoil (21 to 53 inches), were kept separate and each pile was thoroughly mixed. The procedure of filling the boiler-plate cylinders, which were 5 feet in diameter and 5½ feet deep, has been previously described (*47*).

The trees were planted in the spring of 1922. The culture system, consisting of green manuring with buckwheat and rye principally, was uniform in all the cylinders until the spring of 1924, at which time half the cylinders were seeded with a mixture of Kentucky bluegrass and timothy. These cylinders are designated "cylinders under sod." The remaining half of the cylinders were kept under a system of clean cultivation. These are designated "cylinders under cultivation."

A distinction must be noted with respect to the additions of phosphoric acid from 1925 until the end of the experiment in 1927. During these last 3 years of the experiment the cylinders under cultivation received 2.5 g more phosphoric acid than the cylinders under sod. The reason for this is that it was then considered necessary to add equal amounts of organic matter to all the cylinders under cultivation. This was accomplished by growing rye outside the cylinders. For further details the paper by Anthony and Clarke (*2, p. 251*) should be consulted. All trees were allowed to grow without the addition of any mineral fertilizer until the spring of 1925, at which time differential treatment with different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate was commenced.

The schedule of applications of monocalcium phosphate is given in table 1.

The total amount of phosphorus (as P_2O_5) added during the experiment was 1,052.8 g. In addition to the phosphorus carried in the monocalcium phosphate the cover crops contributed approximately 10 g of phosphoric acid. The fertilizer was broadcasted and not mixed with the soil.

TABLE 1.—*Schedule of monocalcium phosphate applications with phosphorus pentoxide equivalent*

Date applied	$CaH_4(PO_4)_2 \cdot H_2O$	P_2O_5 equivalent
	Grams	*Grams*
Apr. 18, 1925	534	300.8
May 3, 1926	267	150.4
June 7, 1926	267	150.4
May 5, 1927	534	300.8
May 18, 1927	267	150.4
Total	1,869	1,052.8

The total precipitation during the period of the experiment was 94.4 inches and, in addition, 2 inches of artificial watering was applied in May 1926, and 1 inch in August 1927.

Samples of the soil of each of the horizons were taken both before and after the completion of the experiment in the manner previously described (*47*). During the period from September 20 to 28, 1927, the trees were dug up and soil samples representative of the three horizons were taken, by the method of successive quartering, from each of the cylinders from which the trees had been removed. These samples were dried at 75° C. and then sieved through a 1-mm sieve (*3*) and stored in glass jars in the dark. Analyses of the trees have already been reported (*46*).

PERCOLATION EXPERIMENTS

Choice of Solvents

The solvent powers of a particular acid, barring reverse or secondary reactions, are related not only to the extent of dissociation of the salts formed and to the dissociation constant of the acid but also to the extent of hydrolysis and to complex ion formation. Moreover, it must be borne in mind that the determination by the aid of weak acids of so-called "available" phosphoric acid (P_2O_5) is not one of dissolution pure and simple but is a mechanism of exchange involving equilibrium (*8, 11*).

With respect to the choice of solvents one might, as some investigators have done (*4, 27, 28*), use only one acid, e. g., hydrochloric acid of different concentrations, thus providing a wide range of pH values. It was decided to use different acids in the present investigation not only because the different solvents chosen provide a wide range of pH values (0.7 to 5.5), but also because they have long been in use by other investigators, and thus permit a comparison of the results obtained with those of others. That fundamental differences with respect to their ability to mobilize phosphoric acid exist is indicated by the difference in their critical concentrations (*10*).

The choice of an acid does not appear to be altogether an arbitrary one for all types of soils, as assumed by some investigators. Laterite soils which are very high in iron oxides are decomposed by hydroxy acids with the formation of acetone and carbon dioxide (*7*), and some hydroxy acids, e. g., citric acid, apparently fail with certain types of calcareous soils (*6*), possibly as a result of a too great reduction of the acidity by the calcium carbonate.

The majority of chemical determinations of the availability of phosphate have been carried out by the "equilibrium" method. The solvents used have included principally (1) citric acid, used by Dyer (*13*); (2) the 0.2 normal nitric acid solvent used extensively by Fraps and his coworkers (*16, 17*); (3) 0.1 or 0.2 normal acetic acid solution, used in France and Germany; and (4) the solvent recently proposed by Truog (*48*), viz, a 0.002 normal sulphuric acid solution buffered with ammonium sulphate. Distilled water has been favored by Schloesing (*40*) and by Wrangell (*52, 53, 54*).

Extraction Procedure

Extraction methods in which the soil is shaken for a definite length of time with the solvent, followed by filtration, washing, and repetition of the procedure with the soil residue, are inconvenient and laborious. Percolation methods which give the same kind of results as the foregoing procedure are much more convenient. In one form or another this method has been used by many investigators. Recently Harper (*25*) has described a simple form of apparatus which has been further modified by the writer, as follows:

A carbon tube is fitted with a platinum cone, above which rests a layer of filter-paper pulp, followed by a layer of 40-mesh leached

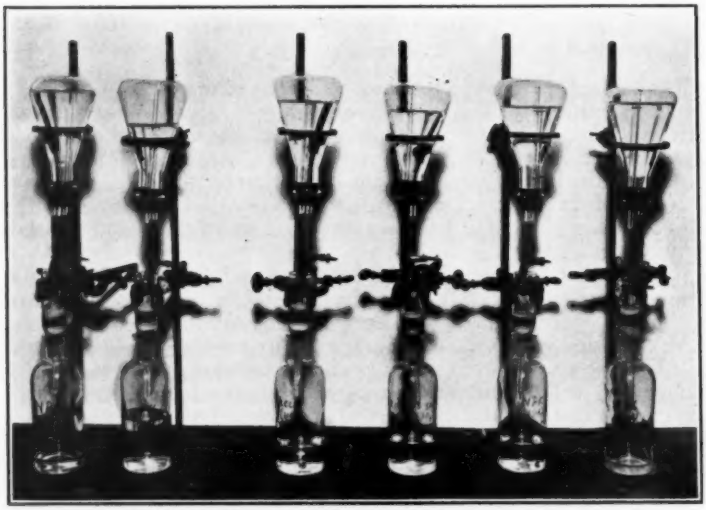

Figure 1.—Battery of the type of percolation apparatus used in the extraction procedure.

quartz sand. Five grams of the soil (moisture-free basis) are then added, gently compacted by tapping, and a layer of 60-mesh acid-washed quartz sand is added. The reservoir consists of a 250-cc flask fitted with air inlet and outlet tube to which is attached a piece of heavy walled rubber tubing fitted with a screw cock. This cock should work freely and be well greased. A battery of such percolation apparatus (fig. 1) is very compact and utilizes little laboratory space. The principle of the method used is one of solution and displacement, and the mechanism is similar to that described by Parker (*34*). The action of a dilute acid on a soil is not constant (*38*), and for this reason the rate of flow can be regulated sufficiently uniformly to give agreement between duplicates by making slight adjustments of the screw cock morning and evening. The extractions were made at laboratory temperature, 75° to 85° F.

Determination of Phosphoric Acid in the Extracts

The four dilute acids used were 0.1 normal acetic (pH 2.8), 0.2 normal nitric (pH 0.7), 0.173 normal citric (pH 2.2), and 0.002 normal sulphuric buffered with ammonium sulphate (pH 3.0). Distilled water (pH 5.5) not freed from carbon dioxide was also used.

The determination of phosphorus in the distilled water and also in the acetic and sulphuric acid leachates was made by the Denigès method (*12*), and in the citric acid and nitric acid leachates by Richards and Godden's modification (*37*) of the Pemberton-Neumann method (*33, 35*). In the latter procedure soluble silica was previously removed by dehydration.

The blue color of the Denigés method is the result of a partial reduction of some of the phosphomolybdate. Within certain limits the color is proportional to the concentration of phosphorus in the solution. The method is very sensitive; 0.001 mg phosphoric acid in 100 cc solution can easily be detected and estimated. The literature relating to the method has been thoroughly reviewed by Zinzadze (*55*), who has developed a technic whereby the blue color is stable over a long period.

EXPERIMENTAL DATA

The quantities in parts per million and absolute (total) amounts of phosphoric acid in the different soil horizons are given in table 2. The results are the mean of duplicate determinations of total phosphoric acid (*3*), which differed by less than 0.005 percent. The results of the percolation experiments with water, sulphuric, acetic, citric, and nitric acids are given in table 3. The volume of each leachate in all cases was 200 cc.

Table 2.—*Parts per million and absolute amounts of total phosphoric acid in the respective horizons before the trees were planted and at the end of the experiment*

Treatment	Total phosphoric acid			Absolute amount total phosphoric acid			
	Surface (0–7 inches)	Subsurface (7–21 inches)	Subsoil (21–53 inches)	Surface (0–7 inches)	Subsurface (7–21 inches)	Subsoil (21–53 inches)	Total (0–53 inches)
	P. p. m.	*P. p. m.*	*P. p. m.*	*Grams*	*Grams*	*Grams*	*Grams*
Soil before trees were planted	1,020	950	1,020	597	1,120	2,744	4,461
Sod:							
Check	1,017	948	1,016	595	1,118	2,733	4,446
NPK	2,796	939	1,017	1,636	1,107	2,736	5,479
PK	2,804	948	1,018	1,641	1,118	2,738	5,497
P	2,819	954	1,018	1,650	1,125	2,738	5,513
Cultivation:							
Check	1,028	955	1,013	602	1,127	2,727	4,456
NPK	2,587	1,045	1,015	1,514	1,232	2,732	5,478
PK	2,623	1,032	1,018	1,535	1,217	2,738	5,490
P	2,645	1,030	1,018	1,548	1,215	2,738	5,501

TABLE 3.—*Phosphoric acid in successive leachings with distilled water, and with sulphuric, acetic, citric, and nitric acids, expressed in parts per million and in absolute amounts*

DISTILLED WATER (pH 5.5)

Description of soil	Phosphoric acid in successive leaching indicated															Total P_2O_5 in all leachings	Absolute amounts
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Soil before trees were planted:	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*[1]	*Grams*
0–7 inches	0	0														0	
7–21 inches	0	0														0	
21–53 inches	0	0														0	
Check (sod):																	
0–7 inches	8.7	[2]														8.7	5.0
7–21 inches	5.3	0														5.3	6.2
21–53 inches	2.5	0														2.5	6.7
Check (cultivation):																	
0–7 inches	10.2	0														10.2	6.0
7–21 inches	5.3	0														5.3	6.3
21–53 inches	2.5	0														2.5	6.7
NPK (sod):																	
0–7 inches	229.0	109.9	77.8	48.1	37.8	28.6	22.9	18.3	17.2	13.7	10.3	9.2	8.9	6.9	5.5	644.1	376.9
7–21 inches	5.9	0														5.9	6.9
21–53 inches	2.7	0														2.7	7.3
NPK (cultivation):																	
0–7 inches	194.5	87.5	56.2	40.3	33.7	22.3	17.4	13.8	11.9	9.8	8.7	7.6	6.5	5.9	5.1	521.2	304.9
7–21 inches	6.8	0														6.8	8.0
21–53 inches	2.7	0														2.7	7.3

[1] Expressed in terms of the dry weight of soil.
[2] Trace.

0.002 N SULPHURIC ACID BUFFERED WITH AMMONIUM SULPHATE (pH 3.0)

Description of soil	Phosphoric acid in successive leaching indicated										Total P_2O_5 in all leachings	Absolute amounts
	1	2	3	4	5	6	7	8	9	10		
Soil before trees were planted:	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*[1]	*Grams*
0–7 inches	0										0	
7–21 inches	0										0	
21–53 inches	0										0	
Check (sod):												
0–7 inches	13.3	7.1	2.7	0							23.1	13.5
7–21 inches	8.7	4.6	3.2	0							16.5	19.5
21–53 inches	3.0	0									3.0	8.1
Check (cultivation):												
0–7 inches	17.8	12.5	7.2	0							37.5	21.9
7–21 inches	8.9	4.2	0								13.1	15.4
21–53 inches	3.0	0									3.0	8.1
NPK (sod):												
0–7 inches	230.8	343.5	230.8	76.0	34.1	6.9	6.4	2.3	(2)	0	930.8	544.6
7–21 inches	9.4	4.1	0								13.5	15.8
21–53 inches	6.4	0									6.4	17.2
NPK (cultivation):												
0–7 inches	234.0	278.2	231.0	109.2	44.2	8.0	6.4	2.2	(2)		913.2	534.3
7–21 inches	11.4	9.4	5.7	5.1	0						31.6	37.3
21–53 inches	6.4	0									6.4	17.2

[1] Expressed in terms of the dry weight of soil.
[2] Trace.

TABLE 3.—*Phosphoric acid in successive leachings with distilled water, and with sulphuric, acetic, citric, and nitric acids, expressed in parts per million and in absolute amounts*—Continued

0.1 N ACETIC ACID (pH 2.8)

Description of soil	Phosphoric acid in successive leaching indicated																				Total P_2O_5 in all leachings	Absolute amounts
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
Soil before trees were planted:	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*[1]	*Grams*
0–7 inches	11.9	4.4	1.1	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	17.4	10.2
7–21 inches	3.4	1.1	(2)	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	4.5	5.3
21–53 inches	3.2	1.1	(2)	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	4.3	11.6
Check (sod):																						
0–7 inches	15.6	6.6	1.1	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	23.3	13.6
7–21 inches	4.6	4.4	1.1	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	10.1	11.9
21–53 inches	3.2	2.7	1.1	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	7.0	18.8
NPK (sod):																						
0–7 inches	357.2	87.0	50.4	50.4	52.7	45.8	41.2	32.1	22.9	14.7	14.0	13.7	12.4	11.5	9.8	8.2	7.3	5.5	4.6	2.3	843.7	493.7
7–21 inches	3.9	1.1	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	5.0	5.9
21–53 inches	3.7	1.1	0	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	4.8	12.9

[1] Expressed in terms of dry weight of soil.
[2] Trace.

1 PERCENT CITRIC ACID (pH 2.2)

Description of soil	Phosphoric acid in successive leaching indicated										Total P_2O_5 in all leachings	Absolute amounts
	1	2	3	4	5	6	7	8	9	10		
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*[1]	*Grams*
Soil before trees were planted:												
0–7 inches	125.4	53.2	39.9	20.9	19.0	17.5	16.2	14.7	13.2	13.0	333.0	194.8
7–21 inches	22.5	13.0	[2]								35.5	41.9
21–53 inches	20.0	8.0	[2]								28.0	75.3
Check (sod):												
0–7 inches	138.0	72.5	45.4	38.2	33.6	30.5	26.3	18.0	10.0	8.0	420.5	246.0
7–21 inches	17.4	7.1	0								24.5	28.9
21–53 inches	21.0	9.8	5.6	0							36.4	97.9
Check (cultivation):												
0–7 inches	142.0	85.6	52.1	33.8	[3] 30.5						53.9	63.6
7–21 inches	28.2	15.3	7.9	2.5	0						37.8	101.7
21–53 inches	21.0	10.0	6.8	0								
NPK (sod):												
0–7 inches	1,096.3	176.7	119.7	87.4	68.4	60.8	60.8	55.0	38.0	22.8	1,785.9	1,045.0
7–21 inches	21.4	10.0	6.2	3.7	0						41.3	48.7
21–53 inches	21.0	11.0	6.0	0							38.0	102.2
NPK (cultivation):												
0–7 inches	902.4	138.7	90.6	70.2	53.2	51.4	45.5	40.4	38.7	25.4	1,456.5	852.2
7–21 inches	38.7	24.3	21.1	20.4	10.2	6.2	0				120.9	142.6
21–53 inches	19.0	10.2	7.1	[2]							36.3	97.6

0.2 N NITRIC ACID (pH 0.7)

Soil before trees were planted:												
0–7 inches	56.0	62.6	45.6	30.4	32.2	30.4	22.4	20.0	19.1	18.0	336.7	197.0
7–21 inches	13.0	8.0	[2]								21.0	24.8
21–53 inches	15.2	8.0	[2]								23.2	62.4
Check (sod):												
0–7 inches	64.6	60.8	57.0	49.4	45.6	38.0	32.3	30.4	30.4	22.8	431.3	252.4
7–21 inches	10.0	6.8	[2]								16.8	19.8
21–53 inches	10.0	7.5	[2]								17.5	47.1
NPK (sod):												
0–7 inches	1,178.0	135.0	91.2	60.8	41.8	39.9	22.9	20.4	20.4	17.1	1,627.5	952.3
7–21 inches	25.2	7.2	[2]								32.4	38.2
21–53 inches	24.7	7.8	[2]								32.5	87.4

[1] Expressed in dry weight of soil.
[2] Trace.
[3] Percolation was not carried further.

DISCUSSION AND INTERPRETATION OF RESULTS

EXTENT OF AND DIFFERENCES IN THE DOWNWARD MOVEMENT OF PHOSPHORUS IN THE TWO CULTURE SYSTEMS AS DETERMINED BY TOTAL PHOSPHORUS CONTENT

The weights of each of the three soil horizons are: Surface 1,290 pounds, subsurface 2,600 pounds, and subsoil 5,930 pounds. The absolute amounts of phosphoric acid can, therefore, be calculated. They are shown in table 2.

The absolute amounts of phosphoric acid in the surface soil of correspondingly treated cylinders under the two systems is very much greater under sod than under cultivation, but the condition is reversed in the subsurface layer. The quantities of residual applied phosphoric acid expected in the surface soil at the end of the experiment can be approximately calculated from the data of table 2 together with the known amounts applied and absorbed by the trees from the added phosphate (*46*). The data so calculated indicate that a downward movement of phosphorus into the subsurface occurred in all the cylinders under cultivation and that no movement into the subsurface occurred under sod. The absolute amounts of phosphoric acid which moved into the subsurface (7 to 21 inches) layer in the cylinders under cultivation were: NPK, 117 g; PK, 113 g; and P, 99 g—equivalent to 11.5 and 9.5 percent of the amounts added in the NPK- and P-treated cylinders.

The difference in the behavior of the cylinders under cultivation with respect to phosphorus movement as well as with respect to the movement of nitrogen (*47*) is interesting. For a better understanding it will be necessary to refer to the distribution and condition of the nitrogen in these same cylinders (*47*). In order to explain the accretion of nitrogen as nonnitric nitrogen in the subsoil (21 to 53 inches) under cultivation (no accretion of nitrogen occurred under sod), three explanations were advanced, viz, (1) assimilation of applied nitric nitrogen by micro-organisms, (2) peptization of organic nitrogen by sodium nitrate in the surface soil and subsequent movement into the subsoil, and (3) the greater root system of the apple trees under cultivation as compared with that under sod.

In searching for an explanation of this movement of phosphorus (and also of nitrogen) in the cylinders under cultivation (but not from under sod) the first explanation given above would a priori be eliminated. The third explanation would account for only a fraction of the phosphoric acid that moved into the subsurface. An additional explanation has recently been suggested, viz, that the living grass roots themselves absorbed and held enough phosphorus as it became available to reduce the amount that moved downward to a negligible quantity. This would be a consequence of greater downward movement of water under cultivation than under sod because there would be less transpiration and, therefore, less upward movement. These causes, no doubt, would be a factor but a minor factor because the phosphoric acid content of the grass in the sod cylinders would account for less than one twenty-fifth of the phosphoric acid that moved into the subsurface layer of the cylinders under the cultivation system.

The second explanation above, viz, that of "peptization" by the organic matter, remains to be considered. There is sufficient experimental evidence (*29, 30, 36*) to show that colloidally dispersed humic

acids (formed by bacterial activity) function in making the soil phosphoric acid more soluble. Ramann (*36*) gives many instances of soils being impoverished in phosphorus under such conditions. Dicalcium phosphate ($CaHPO_4$) is relatively quickly decomposed by humic acids with the formation of calcium humates and free phosphoric acid, the fate of which depends on the amount of sesquioxides present (*30*). The action of the humic acids may be expressed as a "deactivation" of the sesquioxides (*9, 10*).

The relative immobility of phosphorous in the cylinders under sod is in accord with the results obtained in many other long-continued field experiments on the heavier types of soils (*14, pp. 12–124; 43, 49*), where little movement of applied phosphates occurred below plow depth, except where certain salts (*14*) or stable manures (*43*) were added. In orchards under sod, penetration of phosphates in heavy clay soils may not exceed the first inch (*42*). On the other hand, on loosely compacted soils evidence of phosphate movement below the first foot has been obtained (*42, 43, 51*). Phosphoric acid has not been found in the leachates from lysimeter experiments (*22, 31*). In prairie soils a gradual translocation of phosphorus from the subsurface to the surface horizons has been noted (*1*).

CONDITION OF THE PHOSPHORUS

Source of the Phosphorus and of the Iron and Aluminum in the Hagerstown Series

Frear and White (*19*) in an examination of the available phosphoric acid on the grasslands of the Jordan fertilizer plots found only 6.4 percent of the total amount of phosphoric acid present soluble by the Dyer method (one extraction only), and observed that this indicated the absence of apatite. The very small amounts of phosphoric acid dissolved from the original soil by all solvents (table 3) point to the absence of apatite. This is further confirmed by the mineralogical examination of Honess (*44, footnote 2*). Limonite comprises two-thirds of the accessory species in the fine sand fraction (0.2 to 0.1 mm) of the surface soil, and it is probable that the source of phosphorus in this soil is as an impurity in the limonite ($Fe_2O_3nH_2O$), known to contain admixtures of other elements including phosphorus (*5*). Limonite has a high "fixing" capacity for phosphorus (*15*).

During the weathering processes aluminum compounds are in part transferred into various hydrated silicates. The aluminum of this soil is derived from the feldspars, which constitute about 50 percent of the fine-sand fraction and a large proportion of the silt fraction. Other sources of aluminum are the amphiboles and pyroxenes present as accessory species. There is no evidence of the presence of bauxite in this soil (*44*). The iron is present as iron oxides mostly as limonite.

Percolation Data

The leaching experiments were limited to an examination of (1) the original soil before planting (the pH of which was 6.8), (2) the check cylinders under sod and cultivation (the pH of which was 6.65 and 6.60, respectively), (3) the NPK cylinders also under sod and cultivation (the pH of which was 6.50).

The results of the percolation experiments for the three horizons of the foregoing soils are given in table 3.

CONDITION OF THE RESIDUAL PHOSPHORUS IN THE SURFACE HORIZONS

In the case of those solvents having a pH value of 2.2 and below the concentration of the solution with respect to phosphoric acid falls with each successive extraction, and with all solvents tends toward a constant level. At the stage indicated by reduction to this constant level there must exist a phosphate of such low solubility that the amount going into solution at each extraction is independent of the mass present in the soil. But with none of the solvents do the amounts going into solution follow a logarithmic law of decrement in either the phosphate-treated or the untreated soils—a consequence of the existence of more than one phosphate in both soils. In the phosphate-treated soils nitric and citric acids have brought out 60 to 70 percent of the total phosphoric acid in the surface 0 to 7 inches in the first extract, indicating the presence of at least one comparatively easily soluble phosphate which dissolves in proportion to the mass of it present in the soil.

Inasmuch as there is no evidence in these laboratory experiments that the added monocalcium phosphate has effected any significant changes in the solubility of the native phosphorus, we can proceed to ascertain the condition of the added phosphate by comparing the amounts removed by the different solvents after an equal number (ten) of leachings, allowance being made for the amounts extracted from the check cylinder by the respective solvents. The amount dissolved from the unfertilized soil (i. e., the check cylinder) must be taken into consideration because the quantities of phosphoric acid removed by different acids from the check cylinder may bear (as in this experiment) no constant relation to that extracted from the fertilized soil. The results for the NPK cylinder in sod are shown in table 4.

TABLE 4.—*Phosphoric acid* (P_2O_5) *dissolved from the surface soil of the NPK cylinders expressed as a percentage of the residua of the amount applied*

Solvent	Indicated method of calculation	Percentage of residua applied P_2O_5 dissolved
IN SOD		
Distilled water	$\frac{353.1-5.0}{1,039}\times 100=$	33.5
0.1 N acetic acid	$\frac{441.4-13.6}{1,039}\times 100=$	41.2
0.002 N H_2SO_4	$\frac{544.6-13.5}{1,039}\times 100=$	51.1
0.2 N HNO_3	$\frac{952.2-252.4}{1,039}\times 100=$	67.3
0.17 N citric acid	$\frac{1,045.0-246.0}{1,039}\times 100=$	76.9
IN CULTIVATION		
Distilled water	$\frac{285.2-6.0}{917}\times 100=$	30.4
0.002 N H_2SO_4	$\frac{534.3-21.9}{917}\times 100=$	55.9
0.17 N citric acid	$\frac{852.2-246.0}{917}\times 100=$	66.1

The action of a dilute acid upon soil is not a simple action of attack of an easily soluble phosphate followed by a more difficultly soluble phosphate (*23*, *24*), nor, indeed, as already pointed out, is the action of a dilute acid constant (*38*). Rather does extraction with the different weak acids result in a condition of equilibrium which modifies the initial partition of phosphoric acid in the solid and liquid phases. If the amount of phosphoric acid in the liquid phase is sufficiently high the solution will give up phosphoric acid to the soil, and vice versa. There must exist, therefore, a critical concentration (*9*); this critical concentration is different for different acids, low for acetic and nitric acids and high for citric acid (*10*). This critical concentration depends on the phosphorus reserves of the soil and also on the "fixing" power. It is also clear from Russell and Prescott's (*38*) experiments that no definite fraction of the phosphates present is obtained by extraction with dilute acids but only that part expressed as the difference between the amount dissolved and absorbed. For all these reasons it is not possible by such means to separate each of the different phosphorus compounds—to separate, for example, the phosphates of calcium from one another—by treatment of such an adsorption substrate as a soil with different dilute acids, as some investigators have attempted to do.

One may, however, from the data of table 4 conclude that one-third of the residual applied phosphoric acid from the NPK cylinder in sod remained (under conditions of continuous leaching) in a condition soluble in water, one-third has been transformed into somewhat less soluble compounds, and one-third (100 − 67 percent) into very difficultly soluble compounds.

The results for the surface 0 to 7 inches of the phosphate-treated cylinder under cultivation are of the same order of magnitude for the distilled water and sulphuric-acid extractions, viz, 30.4 and 55.9 percent, respectively. But the percentage of the amount residual in the surface layer under cultivation dissolved by citric acid is only 66.1 compared with 76.9 from the correspondingly treated cylinder under sod. This apparently anomalous result will be reserved for later discussion.

The first and second distilled water percolates of the surface 0 to 7 inches of the treated cylinders, both in the sod and in the cultivation systems, contain as much as 13.5 and 7.9 percent of the residual amounts applied during the course of this experiment. This fact suggests that the amounts added may have been more than sufficient to saturate the aluminum and iron hydroxides in these surface layers.

The difficulty soluble compounds into which the soluble phosphate has in part been transformed must be more difficultly soluble than chemically pure ferric phosphate. This may be deduced from the results given in table 5, in which are shown the results of percolation experiments in which chemically pure ferric phosphate, aluminum phosphate, and tricalcium phosphate were each mixed with the original soil in amounts equal to the application of phosphoric acid to the phosphate-treated cylinders. Table 5 gives the percentages of phosphoric acid removed by the different solvents.

TABLE 5.—*Phosphoric acid (P_2O_5) removed by the percolation method from c. p. ferric phosphate, c. p. aluminum phosphate, and c. p. calcium phosphate mixed with the "original" soil*

Solvent	pH	$FePO_4.4H_2O$		$AlPO_4$		$Ca_3(PO_4)_2$	
		Extractions	Removed	Extractions	Removed	Extractions	Removed
		Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Distilled water	5.5	10	0	10	14	10	21
0.002 N sulphuric acid	3.0	10	0	10	32.6	4	100
0.1 N acetic acid	2.8	10	7.9	10	66	6	100
0.17 N citric acid	2.2	8	100	10	100	2	100
0.2 N nitric acid	0.7	15	100	10	100	2	100

The results are similar to those obtained by Heck (*26*) and also are in accord with Gaarder's scheme (*21*). Relative to the comment given on page 333, it may be added that the nearest information that can be obtained with respect to the proportions of the various phosphates existing in the phosphate-treated cylinders is through a comparison of the data of table 5 with those of table 4.

CONDITION OF THE PHOSPHORUS IN THE LOWER HORIZONS

Neither water nor 0.002 N sulphuric acid leached out any phosphoric acid from the original soil. The native phosphorus of these soils is, therefore, not soluble in these solvents. Citric acid removed somewhat more than nitric acid from the lower layers of the original soil.

Percolation with water also removed only minute quantities from the lower horizons of the phosphate-treated (and the untreated) cylinders. One-percent citric acid has dissolved greater quantities of phosphoric acid than 0.2 N nitric acid.

The condition of the phosphoric acid that moved from the surface soil into the subsurface of the phosphate-treated cylinder under cultivation is easily traced. Water removed none of it; Truog's solvent dissolved approximately 20 percent and citric acid about 80 percent of it.

EFFECT OF THE GROWTH OF TREES ON THE SOLUBILITY OF THE NATIVE PHOSPHORUS

More phosphoric acid was dissolved from the surface soil of the check cylinders than from the original soil by all solvents. The increase is very marked with citric and also with nitric acid; the relative increases are 51.2 and 55.4 g, respectively, for the cylinder under sod. Inasmuch as these increments are much greater than the amounts of phosphoric acid contributed in the cover crops (p. 322), the native phosphorus of the soil must have been made more soluble during the growth of the trees.

THE ORGANIC PHOSPHORUS

A number of methods have been proposed for determining the organic phosphorus of soils, but none has yet met the criteria of validity indicated by Frear and White (*20*) in 1911. In these cylinder experiments the organic phosphorus of even the phosphate-treated plots under cultivation as determined by Schollenberger's method

(*41*) represented only about 1 percent of that applied as monocalcium phosphate. The organic phosphorus is, therefore, relatively insignificant as compared to the amount of phosphoric acid applied.

THE "FIXING" CAPACITY OF SOILS

The writer has determined the fixing capacity of the original Hagerstown soil by many of the different methods proposed to determine this property (*15*, *16*, *18*, *25*). It is unnecessary, however, to record the results, inasmuch as no additional information was secured beyond that already recorded—a fact which lends further support to the suggestion (p. 333) that the amounts of phosphate applied during the course of this experiment were more than sufficient to saturate the iron and aluminum hydroxides. The fixing capacity of any one soil type is, as already indicated, a relative and not an absolute property; the amount retained is conditioned not only by the concentration of the phosphate ions and the concentration with respect to other elements, especially those of hydrogen, silica, iron, aluminum, calcium, and magnesium (*9*, *32*), but also by temperature and by time (*15*, *39*, *56*). The results for the same soil will, therefore, differ with the laboratory procedure with respect to the latter factors. If the fixing capacity of different soil types is to be compared, the method described by Demolon and Barbier (*10*) should receive the attention of investigators.

The ability to fix phosphorus cannot be unrelated to the form of the phosphate. Wrangell (*52*) is probably correct in maintaining that the concentration of the soil solution with respect to phosphate ions depends to a greater extent upon the absorbing capacity of the soil than upon the structure and composition of the applied phosphate, for the soil solution has, in all cases examined by Wrangell, a greater concentration with respect to PO_4 ions when treated with tricalcium phosphate (and also rock phosphate) than with monocalcium phosphate. Deductions, however, relative to the availability of phosphates cannot be made from such observations, for, although relatively insoluble phosphates are fixed more slowly than the more soluble phosphates, they are less efficient in supplying available phosphorus to plants (*39*)—a consequence apparently of solid phase feeding.

SUMMARY

The distribution of phosphoric acid (P_2O_5) in three horizons at the conclusion of an experiment lasting 6½ years on a Hagerstown clay loam soil, contained in cylinders planted to apple trees, and treated with different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate, are given in percentage and in absolute amounts.

In all the phosphate-treated cylinders the total (i. e., absolute) amounts of phosphoric acid of the surface soil was greater in the cylinders under sod than in those under cultivation. But in the subsurface layer (7 to 21 inches) the absolute amounts of phosphoric acid present were considerably smaller in the cylinders under sod than in those under cultivation. The downward movement of phosphoric acid into the subsurface layer in the cylinders under cultivation is equivalent approximately to 10 percent of the phosphoric acid added.

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There is no evidence of movement of phosphoric acid into the subsurface layer in the cylinder under sod and none into the subsoil (21 to 53 inches) of the cylinders either under cultivation or under sod.

An explanation advanced is that the differences in the movement phosphorus in the two culture systems is for the most part the result of the mobilizing effect of humic acids.

A simple and compact percolation apparatus is described by the use of which the quantities of phosphoric acid removed in successive leachates from the original soil before the trees were planted and in the soils from untreated and phosphate-treated cylinders by various weak acid solvents were obtained. The results of these extractions are as follows:

In the phosphate-treated cylinders at least one moderately soluble phosphate was present which dissolves in proposition to the mass of it in the soil.

Not a trace of phosphoric acid was found in the leachates from any horizon of the original soil (soil before trees were planted) either by distilled water (pH 5.5) or by 0.002 normal sulphuric acid (pH 3.0).

Thirty-three percent of the phosphoric acid applied to the phosphate-treated cylinders was still soluble in distilled water at the end of the 7-year experiment. One-third remained in a condition of moderate solubility and one-third was converted into basic iron and aluminum compounds more difficultly soluble than ferric phosphate.

There was a conversion into difficultly soluble phosphates of that portion of the applied phosphoric acid that had moved into the subsurface layer in the cylinder under cultivation.

A marked increase was observed in the availability of the phosphoric acid of the soil in the check cylinders over that of the original soil.

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A COMPARISON OF LEPTOSPHAERIA SALVINII AND HELMINTHOSPORIUM SIGMOIDEUM IRREGULARE[1]

By E. M. CRALLEY, *assistant plant pathologist, Arkansas Agricultural Experiment Station,* and E. C. TULLIS, *agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

It has been shown previously (*9*)[2] that *Leptosphaeria salvinii* Catt. on rice has a conidial stage, originally described as *Helminthosporium sigmoideum* Cav. (*3*), and a sclerotial stage, originally described as *Sclerotium oryzae* Catt. (*1, 2*). This fungus occurs on rice in Arkansas, California, Louisiana, and Texas in the United States and has been reported also from Italy, Japan, India, the Philippine Islands, Bulgaria, China, and Cochinchine.

For the past 3 years the writers have observed a similar yet distinctly different fungus on rice in Arkansas, Louisiana, and Texas. This fungus has a conidial stage which is very similar to *Helminthosporium sigmoideum* and a sclerotial stage which is distinctly different from that originally described by Cattaneo as *Sclerotium oryzae*. As no perithecial stage has been observed for the fungus it has been described as *H. sigmoideum* var. *irregulare* (*4*). It was described as a variety rather than as an independent species because of the similarity of the conidia to those of *H. sigmoideum*. The differences are chiefly in the sclerotia.

The symptoms and seasonal development of the disease caused by *Helminthosporium sigmoideum irregulare* are similar to those of the stem rot caused by *Leptosphaeria salvinii* as previously described (*9, 10*) except that in most instances the severity of the disease caused by the former fungus is not so great as that caused by the latter. It is the purpose of the present paper to describe the new fungus more adequately and to compare it with the corresponding stages of *L. salvinii* as previously described.

DESCRIPTION OF HELMINTHOSPORIUM SIGMOIDEUM VAR. IRREGULARE CRALLEY AND TULLIS

Mycelium.—Hyphae white to olivaceous, septate, profusely branched, 2μ to 5μ in diameter. In culture, aerial mycelium usually scanty, submerged mycelium dark. On host, mycelium scanty. Numerous appressoria produced at times.

Sclerotial stage.—Sclerotia very numerous, irregular in outline, black, surface rough, 90μ to 119μ by 268μ to 342μ. Habitat, leaf sheaths and culms of rice, *Oryza sativa* L.

Conidial stage.—Conidiophores dark-colored, septate, erect, simple, 4μ to 5μ by 75μ to 200μ; conidia borne singly on sharp-pointed sterigmata, fusiform, typically three-septate, frequently with germ tubes 2 or 3 times the length of the spore on spores still attached to the conidiophore, intercalary cells densely granular, terminal cells less granular than intercalary cells, 9μ to 12μ by 41μ to 58μ. Habitat, leaf sheaths of rice and on sclerotia floating on water.

[1] Received for publication May 28, 1935; issued October 1935. A joint contribution from the Department of Plant Pathology, Arkansas Agricultural Experiment Station, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Reference is made by number (italic) to Literature Cited, p. 348.

COMPARATIVE MORPHOLOGY OF LEPTOSPHAERIA SALVINII AND HELMINTHOSPORIUM SIGMOIDEUM IRREGULARE

Cultures of *Helminthosporium sigmoideum irregulare* from rice in the United States, Japan,[3] and the Philippine Islands [4] have been studied and all found to be essentially the same. Conidia occurred in all cultures but most abundantly in those from Louisiana and Arkansas in the United States and from Saga, Japan. The sclerotial stage of this fungus also has been reported by Park and Bertus in Ceylon (*6*), and a similar, or perhaps identical, fungus has been described in Japan by Sakurai (*7*) as Sclerotium no. 3.

The sclerotia of *Leptosphaeria salvinii* (*Sclerotium oryzae*) were originally described by Cattaneo (*1*) as being spherical and measuring 350μ to 400μ in diameter. Specimens of cotype material [5] in the mycological collections of the Bureau of Plant Industry, United States Department of Agriculture, shown in figure 1, *A*, *B*, have been examined, and the material agrees in general with the original description except that the sclerotia range from about 190μ to 310μ in diameter. These measurements agree in general with those given by Tisdale (*8*) and Tullis (*9*) for the sclerotia of the fungus in the United States.

The sclerotia of *Helminthosporium sigmoideum irregulare* differ from those of *Sclerotium oryzae* not only in being irregular but also in being distinctly smaller. They measure only 90μ to 110μ by 268μ to 342μ. Morphological differences in the sclerotia of *Leptosphaeria salvinii* and *H. sigmoideum irregulare* are shown in figures 2, *A*, *B*, and 3, *A*, *B*.

Sclerotia of *Leptosphaeria salvinii* and *Helminthosporium sigmoideum irregulare* in culture are shown in figure 4, *A*, *B*. The most striking difference between them is that the sclerotia of *H. sigmoideum irregulare* usually are embedded in the medium and are formed as irregular masses on the radiating strands of the hyphae, whereas sclerotia of *L. salvinii* are spherical or nearly so and are formed individually and as abundantly by the aerial as by the submerged hyphae but not in radiating rows.

The conidia of *Helminthosporium sigmoideum irregulare* are similar in appearance and size to those of *Leptosphaeria salvinii*, as is shown in figures 4, *C*, *D*. Some conidia of the former tend to produce germ tubes from the apex while still attached to the conidiophore, as is shown in figure 4, *E*. In some cases this occurs before cross walls have formed in the spore. Such germ-tube formation has never been observed in the conidia of *L. salvinii* while the spores are still attached to the conidiophores. When grown in artificial culture, frequently one or more septa in the spores of *H. sigmoideum irregulare* are lacking, so that the spores are not so uniformly three-septate as are the conidia of *L. salvinii*. A count was made of the septation of 53 spores of *H. sigmoideum irregulare* in a culture from Arkansas. The results were as follows: Spores without septa, 25; spores with 1 septum, 2; spores with 2 septa, 10; spores typically three septate, 16. Of this number, 50 had germ tubes and 3 had none. Cultures from single conidia of *L. salvinii* with few exceptions have produced only the conidial stage of the fungus, even after

[3] Received from Dr. K. Nakata, Kyushu Imperial University, Fukuoka, Japan.
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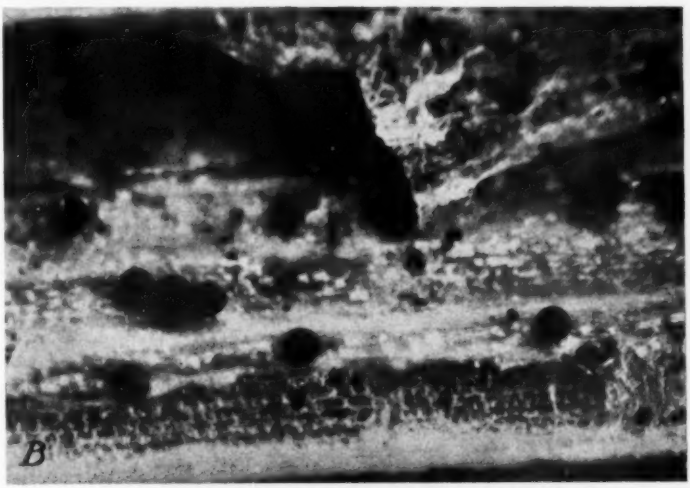

FIGURE 1.—*A*, Reproduction of cotype material of sclerotia of *Leptosphaeria salvinii*, × 1; *B*, enlargement of portion of lower culm in *A*, to show spherical form of the sclerotia. × 30.

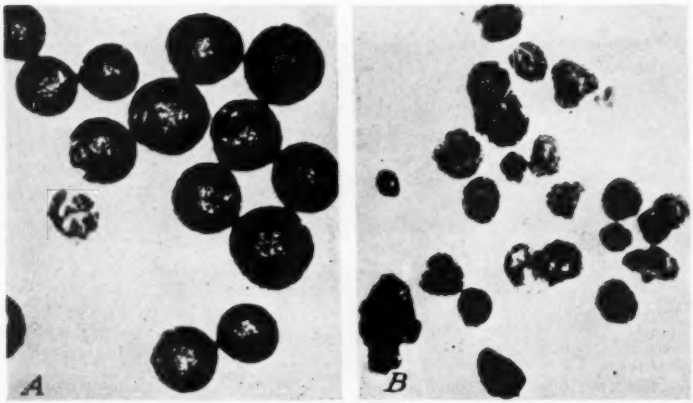

FIGURE 2.—Sclerotia of *Leptosphaeria salvinii* (*A*) and *Helminthosporium sigmoideum* (*B*) from culms of Blue Rose rice. × 40.

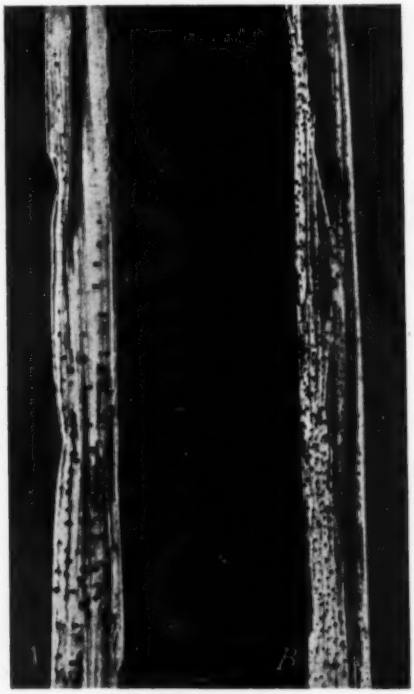

Figure 3.—Stem-rot-diseased culms of Supreme Blue Rose rice: *A*, Sclerotia of *Leptosphaeria salvini*, × 3; *B*, sclerotia of *Helminthosporium sigmoideum irregulare*. × 3.

numerous successive transfers, whereas cultures from single conidia of *H. sigmoideum irregulare* consistently have produced sclerotia at first and conidia later.

Numerous attempts have been made to determine whether typical spherical sclerotia of *Leptosphaeria salvinii* might be produced by transfers of sclerotia or conidia of *Helminthosporium sigmoideum irregulare* or from isolations of the fungus from diseased plants, but the attempts have given negative results. Other experiments were made in which *H. sigmoideum irregulare* was grown on various media, including sterilized rice straw and rice-straw ground and incorporated in agar of various kinds, in attempts to produce the sclerotia typical

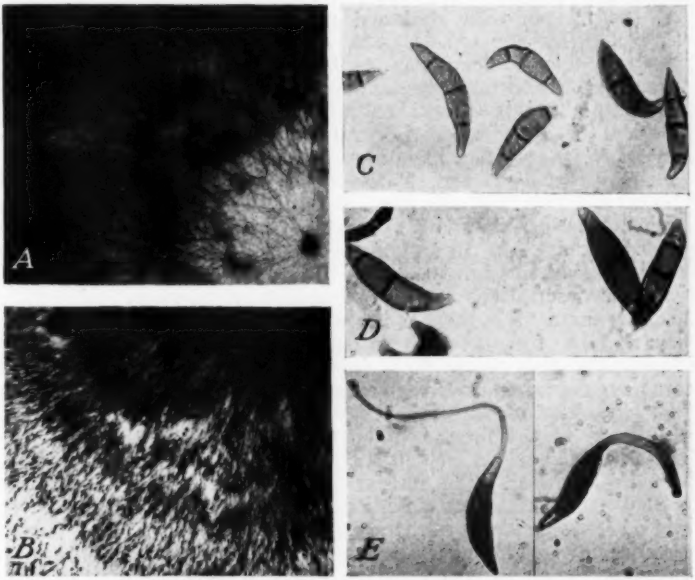

FIGURE 4.—*A*, Portion of a colony of *Leptosphaeria salvinii* growing on corn-meal agar. × 1.5; *B*, portion of a colony of *Helminthosporium sigmoideum irregulare* growing on corn-meal agar. × 1.5; *C*, conidia of *H. sigmoideum irregulare*. × 320; *D*, conidia of *L. salvinii*. × 400; *E*, conidia of *H. sigmoideum irregulare* with germ tubes. × 320.

of *L. salvinii*. In all cases, regardless of the appearance of the sclerotia on these media, no sclerotia typical of *L. salvinii* were produced when transferred back to corn-meal agar. *H. sigmoideum irregulare* produced conidia readily on an agar composed of 1 percent pectin and 4 percent agar.

In a previous paper (*10*) the writers described the production of conidia by sclerotia of *Leptosphaeria salvinii* floating on water. *Helminthosporium sigmoideum irregulare* also produces conidia under these conditions. Sclerotia from Louisiana and from plants infected with the culture received from Saga, Japan, were used. The first conidia were produced in 7 and 20 days, respectively, and conidial formation continued for 5 months, at which time the experiment was discontinued. During this period as many as 40 conidia were pro-

duced by some of the sclerotia. Individual sclerotia of *L. salvinii* under similar conditions in the same period have produced as many as 75 conidia.

Since this manuscript was prepared for publication the attention of the writers has been called to a book by Nakata (*5*) on crop diseases,[6] published in Tokyo, September 15, 1934. In the discussion of these diseases, Nakata describes as *Helminthosporium sigmoideum* Cav. the fungus with irregular sclerotia, which the writers have described (*4*) as *H. sigmoideum irregulare;* and he describes as *H. sigmoideum* var. *microsphaeroides* Nakata the fungus with spherical sclerotia, which has been shown to be *Leptosphaeria salvinii* Catt.

As previously shown (*10*), and again pointed out in the present paper, the fungus with spherical sclerotia, *Leptosphaeria salvinii* Catt., has *Sclerotium oryzae* Catt. as its sclerotial stage and *Helminthosporium sigmoideum* Cav. as its conidial stage. On the basis of the descriptions and measurements of sclerotia and conidia given by Nakata (*5, p. 24*), it is evident that his *H. sigmoideum microsphaeroides* is identical with the sclerotial and conidial stages of *L. salvinii.* Furthermore, the writers have secured a culture from Institut voor Shimmelcultures at Baarn, Netherlands, which had been supplied by Nakata. This culture was labeled *Sclerotium oryzae,* and in all essentials is identical with cultures of *L. salvinii* from the United States, India, and the Philippine Islands. It is evident, therefore, that the name of this fungus should be *L. salvinii* Catt.

From the descriptions and measurements of sclerotia and conidia given by Nakata (*5, p. 22*) for the fungus with irregular sclerotia, it is clear that the fungus which he has included under the name *Helminthosporium sigmoideum* Cav. in reality is the fungus which the writers have described as *H. sigmoideum* var. *irregulare* (*4*). Cultures of "*H. sigmoideum*" supplied the writers by Nakata are identical with cultures of the fungus which they have described as *H. sigmoideum* var. *irregulare.* Even the characteristic germ tubes of the conidia are identical. The formation of germ tubes by the conidia while still attached to the conidiophores, is a characteristic of the conidia of *H. sigmoideum* var. *irregulare* not found in those of *Leptosphaeria salvinii.* These germ tubes have been observed by the writers on numerous occasions on conidia from the cultures received from Nakata as "*H. sigmoideum.*" It is evident, therefore, that the fungus which Nakata has called *H. sigmoideum* Cav., is identical with that which the writers have described as *H. sigmoideum* var. *irregulare* and that the true *H. sigmoideum* Cav. is the conidial stage of *Leptosphaeria salvinii* Catt.

According to the above interpretation, the synonymy for these two fungi is as follows:

(1) *Leptosphaeria salvinii* Catt. Synonyms for conidial and sclerotial stages:
Helminthosporium sigmoideum Cav.
Helminthosporium sigmoideum Cav. var. *microsphaeroides* Nakata
Sclerotium oryzae Catt.

(2) *Helminthosporium sigmoideum* Cav. var. *irregulare* Cralley and Tullis. Synonym:
Helminthosporium sigmoideum Nakata, not Cav.

[6] Saburo Katsura has kindly translated portions of the text dealing with the sclerotial diseases of rice.

PATHOGENICITY OF HELMINTHOSPORIUM SIGMOIDEUM IRREGULARE

Inoculation experiments were conducted to test the pathogenicity of *Helminthosporium sigmoideum irregulare* and also to test its ability to produce conidia on the host.

Inoculation tests were made in the laboratory and in the greenhouse. The first inoculations were made by placing bits of mycelium on agar adjacent to single sterile seedlings of Supreme Blue Rose rice growing on corn-meal agar in test tubes. The results of these inoculations are shown in table 1.

Ninety-day-old plants of Supreme Blue Rose growing in the greenhouse in stoneware jars were inoculated in duplicate with 8 cultures. Inoculations with 7 cultures were made by inserting bits of mycelium on agar under the outer leaf sheaths. Inoculation with culture 8 was made by scattering sclerotia on the surface of the water. The source of the cultures and the results of these inoculations are shown in table 2.

TABLE 1.—*Results of inoculation of rice seedlings growing under aseptic conditions on corn-meal agar in test tubes with 6 cultures of Helminthosporium sigmoideum irregulare*

Source of culture	Plants inoculated[1]	Plants infected	Source of culture	Plants inoculated[1]	Plants infected
Japan:	*Number*	*Percent*	United States:	*Number*	*Percent*
Kumamoto	4	100	Louisiana (Crowley)	4	100
Okayama	4	100	Arkansas	10	100
Hiroshima	4	100	Control, 12 plants not inoculated		0
Saga	4	100			

[1] The conidial stage was not observed on any of the inoculated dead plants.

TABLE 2.—*Results of inoculation of Supreme Blue Rose rice plants in the greenhouse with sclerotia of the various cultures of Helminthosporium sigmoideum irregulare, 1932–33*

Culture No.	Source	Plants inoculated	Plants infected		Culture No.	Source	Plants inoculated	Plants infected	
	Japan:	*Number*	*Number*	*Percent*		Japan:	*Number*	*Number*	*Percent*
1	Kumamoto	23	3	13.0	6	Fukuoka	25	0	.0
2	Okayama	28	1	3.6	7	Okayama	71	12	16.9
3	Hiroshima	67	[1]19	28.4	8	Arkansas	232	[1]99	42.7
4	Saga	61	[1]5	8.2					
5	Louisiana	212	112	52.8		Average			34.9
						Control, 61 plants not inoculated		0	.0

[1] Typical conidia of *H. sigmoideum irregulare* were produced on these infected plants.

Nearly 35 percent of the total number of plants inoculated became infected. As compared with the rather high percentage of infection obtained from the cultures from Arkansas and Louisiana—42.7 and 52.8, respectively—the percentages of infection from those obtained from Japan were rather low and irregular, ranging from 0 to 28.4. However, the number of plants inoculated with these cultures was not

large, and the cultures may possibly have been rather old. The results demonstrate, however, that *Helminthosporium sigmoideum irregulare* is able to infect healthy plants and to produce in and on them sclerotia and conidia similar to the forms used in the inoculations. The fungus was reisolated from the diseased plants.

SUMMARY

A description of *Helminthosporium sigmoideum* var. *irregulare* is presented.

A comparison of the morphology of *Leptosphaeria salvinii* and *Helminthosporium sigmoideum* var. *irregulare* is given and the pathogenicity of the latter on rice is shown.

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COMPARATIVE TOXICITY OF ANABASINE AND NICOTINE SULPHATES TO INSECTS [1]

By JOSEPH M. GINSBURG, *biochemist in entomology*, JOHN B. SCHMITT, and PHILIP GRANETT, *research assistants, New Jersey Agricultural Experiment Station*

HISTORICAL REVIEW

Within the last few years the Government of the Union of Soviet Socialistic Republics has placed anabasine sulphate on the American market as a new contact poison selling at a price considerably lower than nicotine sulphate. Anabasine is an alkaloid present in the stems and leaves of *Anabasis aphylla* L., a member of the Chenopodiaceae. It is somewhat similar to nicotine in its structural formula and has the same empirical formula ($C_{10}H_{14}N_2$). Anabasine is considered an isomer (*7*)[2] of nicotine. Like nicotine, it can be readily extracted from the plant and converted into a sulphate.

Aphylla is only one of a large number of species belonging to the genus *Anabasis*. It is a perennial weed growing (*10*) in northern Africa, Russia, Armenia, and in the neighboring countries. Attempts are at present being made to cultivate it on the North American continent. The amount of alkaloid varies from a fraction of 1 percent in old twigs and thick leaves to more than 2 percent in young succulent twigs and young growing leaves. The commercial product anabasine sulphate contains approximately 40 percent of total alkaloids, of which about 70 percent is anabasine (*1*, *7*), the remainder being lupinine, other higher alkaloids, and miscellaneous plant material.

It may be of interest that not long before Orechoff and Menschikoff (*8*) had isolated anabasine from the plant, Smith (*9*) synthesized, in 1930, the alkaloid β-pyridyl-α-piperidine, which he called "neonicotine" and which he later (*11*) found to be chemically the same as the natural anabasine. The only difference between the two is that neonicotine is optically inactive while anabasine is levorotatory. Smith, Richardson, and Shepard (*12*) found neonicotine as toxic to *Aphis rumicis* L. as nicotine.

Garman (*2*, *3*, *4*) reports that both anabasine and anabasine sulphate are more toxic to aphids than are nicotine and nicotine sulphate. In his experiments, dilutions of 1 pint of either anabasine sulphate or nicotine sulphate to 100 gallons of water produced equally high control of the white apple leafhopper. On the other hand, Campbell, Sullivan, and Smith (*1*) found anabasine less toxic than nicotine to culicine mosquito larvae.

[1] Received for publication May 17, 1935; issued October 1935. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Entomology.

[2] Reference is made by number (italic) to Literature Cited, p. 354.

EXPERIMENTAL WORK

In May 1931 the senior writer tested the toxicity to honeybees of anabasine sulphate received from the Amtorg Trading Corporation. Applied as a contact spray, concentrations of 0.2 and 0.1 percent killed 100 percent of the bees within 24 hours. When the properties of anabasine, as an internal poison, were tested by allowing the bees to feed on honey containing 0.2 percent of anabasine sulphate, only about 10 percent died within the same period. On the other hand, in a previous publication (*5*) the senior writer reported high percentages of kill of bees fed on honey containing 1 part of nicotine, in the form of nicotine oleate, to 3,200 parts of honey mixture. These preliminary results suggested that anabasine is much more effective as a contact poison than as a stomach poison.

Experiments with anabasine sulphate were resumed during the summer of 1934. Since nicotine is largely used in agricultural sprays to control aphids, the major part of this investigation was devoted to comparing the aphicidal properties of the two insecticides. The samples used in these tests contained 40.5 percent and 40 percent total alkaloids for nicotine sulphate and anabasine sulphate, respectively, as stated in the chemical analysis submitted by the manufacturers.

The tests with various dilutions of anabasine sulphate and nicotine sulphate were conducted on several species of aphids, on silk moth larvae, and on grasshoppers. The toxicity to insects was determined by methods previously described (*6*); 90 percent kill or higher was considered efficient control.

TESTS ON APHIDS

The following species of aphids were used in these tests: *Aphis pomi* De G., on apple; *Aphis rumicis*, Lin. on nasturtium; *Macrosiphum rosae* Lin. on roses; *Macrosiphoniella sanborni* Gill and *Rhopalosiphum rufomaculata* Wils., on chrysanthemum. In order to spread and wet efficiently, each spray solution had added to it 0.2 percent of coconut-oil soap. Several series of tests were run for each dilution. The average results are reported in the tables.

Laboratory Tests

Check tests with the wetting agent alone have shown that 0.2 percent of soap killed approximately 14 percent of the green apple aphids (*Aphis pomi*), 21 percent of the nasturtium aphids (*A. rumicis*), and 13 percent of *Macrosiphum rosae*. The concentrations of the spray mixtures ranged from 1 pint to one twenty-fourth of a pint per 100 gallons. The high dilutions, although not practical, were necessary in order to evaluate the differences between the toxicity of the two alkaloids. The results from tests on the aphids are set forth in table 1. A comparison of the results with apple and nasturtium aphids reveals no distinct differences in toxicity of the two insecticides in concentrations of one-half or one-third of a pint per 100 gallons, the percentage of kill being 90 or better in each case. At concentrations of one-sixth and one-twelfth of a pint the results were

decidedly higher, on both species of aphids, with anabasine sulphate than with nicotine sulphate. The rose aphids were more resistant to both anabasine and nicotine, 1 pint of anabasine sulphate being required per 100 gallons to produce about a 90 percent kill; the percentages of dead rose aphids were consistently higher with anabasine sulphate than with nicotine sulphate.

TABLE 1.—*Toxicity tests with anabasine and with nicotine sulphates on green-apple aphids (Aphis pomi), nasturtium aphids (A. rumicis), and rose aphids (Macrosiphum rosae)*

Insecticide in 100 gallons of spray [1] (pints)	Green-apple aphids		Nasturtium aphids		Rose aphids	
	Total insects	Dead after 24 hours	Total insects	Dead after 24 hours	Total insects	Dead after 24 hours
Anabasine sulphate:	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1					321	90.3
½	888	94.8			436	78.4
⅓	1,342	97.6	511	95.8	334	66.0
⅙	618	93.4	322	90.9	608	60.0
1/12	1,098	66.4	447	78.0		
1/24	745	31.0	370	53.7		
Nicotine sulphate:						
1					372	87.6
½	1,200	97.3			336	59.0
⅓	1,736	90.2	272	92.6	471	52.4
⅙	435	82.3	272	86.0	383	48.5
1/12	1,982	58.9	788	66.7		
1/24	686	38.1	273	48.7		
Check, 0.2 percent of soap: 0	1,338	14.7	716	21.4	301	13.0

[1] A concentration of 1 pint of insecticide per 100 gallons of spray represents an approximate dilution of the alkaloids of 1 to 2,000.

GREENHOUSE TESTS

Several beds of chrysanthemum plants, in a commercial greenhouse, were divided into two parts and sprayed with the two insecticides on August 2, 1934. In each case only one concentration, namely, one-third of a pint of insecticide per 100 gallons of spray solution, was used. The plants were infested with two different species of aphids, the black (*Macrosiphoniella sanborni*) and the green (*Rhopalosiphum rufomaculata*). After approximately 24 hours, gross observations showed a practically complete kill of the black aphids and an incomplete kill of the green aphids with each of the spray mixtures. In order more closely to verify these differences, several plants were removed from each plot, and counts of live and dead aphids were made. The results, set forth in table 2, show 100-percent control of black aphids with both spray mixtures. The control of green aphids disclosed striking differences in favor of the anabasine sulphate, the actual percentages killed being 87.8 for anabasine sulphate and 32.1 for nicotine sulphate. The experiments were repeated on August 8 on different chrysanthemum plants in the same greenhouse. Only the green aphids were counted. The results (table 2) again show high kill with anabasine sulphate and low kill with nicotine sulphate. It appears, therefore, from these results that anabasine possesses higher toxicity to this species of aphids than does nicotine.

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TABLE 2.—*Toxicity tests with anabasine and with nicotine sulphates*[1] *on two species of aphids, Macrosiphoniella sanborni and Rhopalosiphum rufomaculata, infesting chrysanthemum plants*

Insecticide tested	Date of spraying	Black aphids, *Macrosiphoniella sanborni*		Green aphids, *Rhopalosiphum rufomaculata*	
		Total insects on 4 plants	Dead after 24 hours	Total insects on 4 plants	Dead after 24 hours
		Number	*Percent*	*Number*	*Percent*
Anabasine sulphate	Aug. 2, 1934	297	100	296	87.8
Nicotine sulphate	do	173	100	205	32.1
Anabasine sulphate	Aug. 8, 1934			31	87.1
Nicotine sulphate	do			329	28.8

[1] One-third pint of insecticide per 100 gallons of spray.

TESTS ON CHEWING INSECTS

LABORATORY TESTS ON SILK MOTH LARVAE

The silk moth larva (*Bombyx mori* L.) has proved to be a satisfactory insect-indicator for testing stomach poisons in this laboratory. A comparison of the toxicity of anabasine and nicotine sulphates to healthy silk moth caterpillars was, therefore, made. Because of the scarcity of the insects at the time of testing, only 10 insects, all in the fourth instar, were used for each single test. In order to eliminate volatility of the alkaloids, 0.1 percent of a neutral wetting agent[3] was added to each spray mixture, instead of soap to produce efficient spreading.

The results, presented in table 3 show that concentrations of 1 quart of nicotine sulphate to 100 gallons of water gave a 100-percent kill in 2 days, whereas anabasine sulphate gave a 30-percent kill in 3 days. In concentrations of 1 pint to 100 gallons, nicotine sulphate gave about 95-percent kill and anabasine sulphate only 15-percent kill in 3 days.

TABLE 3.—*Toxicity tests with anabasine and with nicotine sulphates on silk-moth larvae, using 10 larvae per test*

Insecticide tested	Insecticide per 100 gallons of spray[1]	Dead—		
		After 1 day	After 2 days	After 3 days
	Pints	*Percent*	*Percent*	*Percent*
Nicotine sulphate	2	90	100	
Do	1	40	90	[2] 100
Do	1	30	70	[2] 90
Anabasine sulphate	2	0	10	[3] 30
Do	1	0	0	[3] 10
Do	1	0	10	[3] 20
Check (0.1 percent of arescap)	0	0	0	0
Do	0	0	0	0

[1] See note, table 1, for approximate dilution of the alkaloids. [2] Average, 95. [3] Average, 15.

[3] Arescap obtained from Monsanto Chemical Co., St. Louis, Mo.

Laboratory Tests on Grasshoppers

Adults of a short-horned grasshopper (*Melanoplus femur-rubrum*, De G.), commonly called "red-legged locust", collected from a meadow near the college campus, were transferred to cages and fed on young potted tomato plants, sprayed 1 hour previously, with various concentrations of nicotine sulphate and anabasine sulphate. Twenty insects were used for each test. Each spray solution contained 0.1 percent of the neutral wetting agent (Arescap). The tomato plant is evidently very palatable to this species of grasshopper, since the insects rapidly devoured the leaves on the check plants, sprayed only with 0.1 percent of Arescap, so that the plants had to be renewed once during the 3 days of testing. The feeding on the plants sprayed with the two insecticides, however, was rapidly retarded after the first day especially on those sprayed with nicotine sulphate, and in neither case was the renewal of plants necessary.

The results, presented in table 4, show that nicotine sulphate is decidedly more toxic to this chewing insect than is anabasine sulphate. At concentrations of 1 pint and 1 quart per 100 gallons of spray, nicotine sulphate produced 80- and 90-percent kill, respectively, as compared with 40- and 60-percent mortality obtained with the same concentrations of anabasine sulphate.

Table 4.—*Toxicity tests with anabasine and with nicotine sulphates on grasshoppers, in which 20 insects per test were used*

Insecticide tested	Insecticide per 100 gallons of spray	Dead after 3 days
	Pints	*Percent*
Nicotine sulphate	1	80
Do	2	90
Anabasine sulphate	1	40
Do	2	60
Check, 0.1 percent of Arescap	0	15

SUMMARY AND CONCLUSIONS

Laboratory and greenhouse tests were conducted with anabasine sulphate and nicotine sulphate on several species of aphids, silk moth larvae, grasshoppers, and honeybees. The results definitely show that:

Anabasine sulphate equals or excels nicotine sulphate in toxicity to *Aphis rumicis*, *A. pomi*, and *Macrosiphoniella sanborni* and is decidedly more toxic to *Rhopalosiphum rufomaculata* and *Macrosiphum rosae*.

Anabasine sulphate possesses very little toxicity as a stomach poison against silk moth larvae, while nicotine sulphate proved highly toxic to this insect.

Anabasine sulphate was decidedly less toxic to grasshoppers, applied as a stomach poison, than was nicotine sulphate.

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THE TOXICITY OF OPTICALLY ACTIVE AND INACTIVE DIHYDRODEGUELINS [1]

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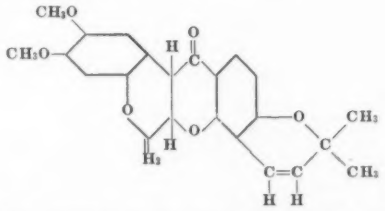
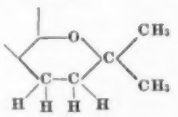
INTRODUCTION

During a study of toxic constituents of derris root other than rotenone, Haller and LaForge prepared two dihydrodeguelins, one optically active and the other optically inactive (*7*).[2] Since the physiological action of a compound may depend on its configuration as well as on its chemical constitution, it was considered of value to study these two derivatives toxicologically.

Although Haller and LaForge found the "deguelin concentrate"—the amorphous residue left after separation of the rotenone and toxicarol present in petroleum ether extract of derris root—to be strongly levorotatory, they were unable to obtain crystalline active deguelin from it. Thus, the inactive modification was the only form available for comparative purposes. This compound was prepared by these workers by treatment of deguelin concentrate with alkali. However, the sample of inactive deguelin (melting point 171° C.) used in the following study was prepared by Clark (*1*). Its constitution (*2*) is expressed by the formula:



By catalytic hydrogenation of deguelin concentrate Haller and LaForge (*7*) obtained levodihydrodeguelin (melting point 155°–156° C.). After separating the portion that crystallized out, they isolated as the inactive form (melting point 171°) the dihydrodeguelin remaining in the mother liquor by alkali treatment and subsequent crystallization. The structure of these dihydro derivatives differs from that of deguelin only by saturation with hydrogen at the double bond in the chroman system, thus:



[1] Received for publication June 27, 1935; issued October 1935.

[2] Reference is made by number (italic) to Literature Cited, p. 360.

It is the presence of this system instead of the benzofuran system which differentiates these compounds from their isomers, rotenone and dihydrorotenone (*8*).

EXPERIMENTAL PROCEDURE

The method used in studying the toxicity of these compounds has been described previously (*3*). Goldfish of the same lot, weighing from 2.7 to 3.7 g each, were used in all the tests, and a constant temperature (27° C.) was maintained. For comparative purposes tests were also made with rotenone. In addition, because a definite relationship has been established between certain dihydro derivatives of rotenone and their parent compounds, it was thought of interest to make tests with deguelin.

RESULTS

The toxicological data are given in table 1. The survival-time curves and the velocity-of-fatality curves, which are plotted from these data, are given in figures 1 and 2.

TABLE 1.—*Toxicity of rotenone, deguelin, and active and inactive dihydrodeguelins to goldfish at 27.0° ±0.2° C.*

Compound	Concentration	Fishes used	Mean length of fishes	Mean weight of fishes [1]	Mean survival time	Mean $\frac{1,000}{\text{survival time}}$
	Milligram per liter	*Number*	*Millimeters*	*Grams*	*Minutes*	
Rotenone	0.900	14	46	2.9	127	8.06
	.600	16	48	3.3	131	7.98
	.500	13	48	3.3	144	7.14
	.400	12	48	3.3	149	6.86
	.300	12	49	3.4	154	6.93
	.200	13	46	2.9	192	5.63
	.150	13	48	3.3	229	4.78
	.120	11	46	2.9	250	4.20
	.100	27	47	3.1	280	3.91
	.075	13	48	3.3	377	2.76
	.050	12	48	3.3	540	1.95
	.030	12	50	3.7	780	1.27
Deguelin	.700	9	48	3.3	152	6.91
	.400	12	47	3.1	168	6.03
	.300	11	49	3.5	181	5.73
	.250	34	48	3.3	204	5.25
	.200	46	48	3.3	238	4.61
	.150	34	47	3.2	299	3.75
	.120	11	48	3.3	380	3.17
	.100	14	48	3.3	455	2.36
Active dihydrodeguelin	.900	10	47	3.0	126	8.13
	.500	12	45	2.7	132	7.72
	.400	15	45	2.7	147	6.90
	.300	11	47	3.0	154	6.84
	.250	16	47	3.1	159	6.46
	.200	27	48	3.3	199	5.32
	.150	13	47	3.1	242	4.35
	.120	11	46	2.9	262	4.21
	.100	28	49	3.5	352	3.10
	.075	11	49	3.5	474	2.24
	.030	9	[2]	[2]	[2]	[3] 3.55
Inactive dihydrodeguelin	.900	10	47	3.1	159	6.36
	.600	13	47	3.1	181	5.68
	.500	11	48	3.3	213	5.15
	.400	13	49	3.4	224	4.94
	.300	12	47	3.0	269	4.12
	.240	12	49	3.5	295	3.71
	.200	13	47	3.1	392	2.98
	.150	9	48	3.3	496	2.24
	.100	12	46	3.0	930	1.17

[1] Estimated from length, which measurement excludes the tail.
[2] Fishes not measured but of same approximate size.
[3] 5 fishes survived 17 hours; 4 fishes were apparently unaffected after 48 hours. These figures are only approximate, since a large number of fishes would be required to give an accurate mean value. The reciprocal of the survival time of a fish surviving the test is taken as zero, since the reciprocal of any survival time longer than the test would be negligibly small.

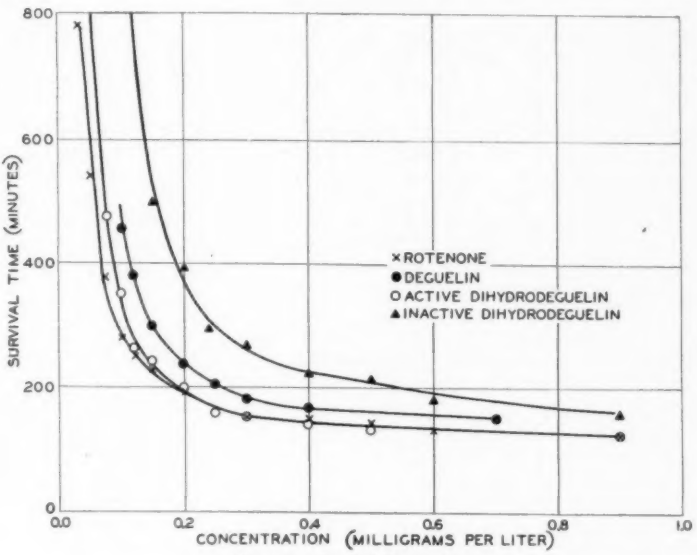


FIGURE 1.—Survival-time curves.

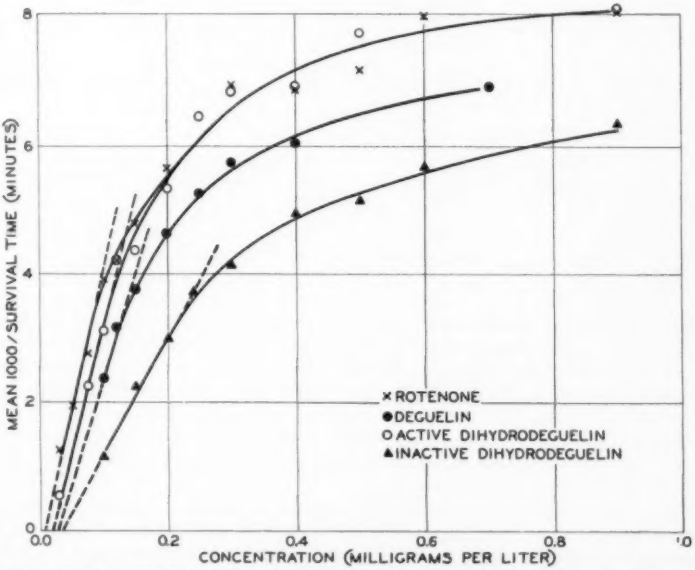


FIGURE 2.—Velocity-of-fatality curves. Straight lines indicate approximation of that portion of the curve corresponding to greatest rate of increase of velocity of fatality with increase in concentration.

DISCUSSION

Comparative data obtained from these curves are given in table 2. In figure 2 the straight line, which is an approximation of that portion of the curve corresponding to the greatest rate of increase in the velocity of fatality with increase in concentration, is prolonged to cut the X-axis at a point designated "c_0"; the slope of this line is designated "tan θ", and its measurement is expressed in the dimensions of both coordinates. In this way values are obtained, respectively, for the theoretical threshold of toxicity, or the concentration below which the substance does not cause death, and for the maximum rate of increase of the velocity of fatality with increase in concentration. In a previous study (*5*) of nine substances with relatively low thresholds a point on the survival-time curve called the "minimum *ct* product", $(ct)_m$, was found to correspond very closely to the upper end of this straight line, and this fact has been employed in locating the individual lines. Beyond the arching portion of either the survival-time curve or the velocity-of-fatality curve in the direction of higher concentrations it approaches a region of constant velocity; that is, the curve approaches the horizontal. To obtain values for comparative purposes, the region in which the survival times corresponding to concentrations, one of which is double the other, do not differ by more than 5 percent is chosen arbitrarily as the minimum survival time and designated "t_0." The values given for t_0 in the table are approximated from the respective survival-time curves.

TABLE 2.—*Relative toxicity at 27° C. of rotenone, deguelin, and optically active and inactive dihydrodeguelins*

Compound	c_0	tan θ	$\sqrt{\frac{\tan\theta}{c_0}}$	t_0	c_m	t_m	$(ct)_m$	Relative toxicity according to $(ct)_m$
	Milligram per liter	*Liter per milligram per minute*		*Minutes*	*Milligram per liter*	*Minutes*	*Milligram minutes per liter*	
Rotenone	0.01	0.044	2.1	120	0.085	320	27.2	1.00
Deguelin	.03	.033	1.1	130	.135	329	44.4	.614
Active dihydrodeguelin	.02	.040	1.4	120	.110	302	33.2	.819
Inactive dihydrodeguelin	.035	.018	.72	140	.220	333	73.3	.371

The minimum product of concentration and time, $(ct)_m$, was suggested in a previous paper (*5*) as a suitable criterion for the comparison of toxicity, since it gives a relative value at the point of greatest efficiency of the toxic substance with respect to concentration and time. The value is determined from the survival-time curve; its probable error is less than 7 percent of the mean survival time. Its coordinates, c_m and t_m, are given in order that the point can be located on the curve.

It is apparent from columns 3 and 9 of table 2 that, compared according to either of the methods in which the toxicity of a substance is considered only at its highest efficiency with respect to concentration and time, these compounds have the following descending order of toxicity: Rotenone, active dihydrodeguelin, deguelin, and inactive dihydrodeguelin. Compared according to the values ob-

tained for the respective thresholds of toxicity, c_0, this order still obtains. It must be emphasized, however, that these values are obtained under theoretical conditions and may have no real significance beyond showing the order of toxicity at very great dilutions. Also they are approximate only, since many more observations would be necessary to distinguish between the closer values with accuracy. Similarly, the values given for the minimum survival time, t_0, are only approximate and for the region of the curves as indicated above. There is no evidence that these values would differ if the survival times were compared at concentrations above those given at the same relative portions of their curves; the indications are that they would be close together. An extrapolative procedure such as was used to obtain the theoretical threshold values—that is, plotting the reciprocal of the concentration against survival time and using the points corresponding to the minimum *ct* products as the upper limits of the straight lines—does indicate that all the compounds have a time tolerance of 100 ± 5 minutes; that is, an infinitely high concentration requires 100 minutes to kill, if it is assumed that no other factor has entered to change the type of toxic action.

It is also to be noted that the figure obtained by comparison of deguelin with rotenone agrees well with the value obtained in a previous study (*4*) with another group of goldfish. According to the formula of Powers (*9*), $\sqrt{\frac{\tan \theta}{c_0}}$,[3] which was used in that study for comparing toxicity, deguelin had 0.56 the toxicity of rotenone. In the present study, according to the same formula and against a much more resistant group of goldfish, the relative value, or ratio, is nearly the same, 0.52. As determined by a comparison of the slopes of the theoretical velocities of fatality, the ratios are, respectively, 0.79 and 0.75. According to the author's method of comparing minimum *ct* products, the ratios are 0.55 and 0.61. For a practical comparison, as explained in a previous paper (*5*), the last figures may be accepted; that is, deguelin has 0.6 the toxicity of rotenone.

Optically active dihydrodeguelin has essentially the same toxicity as rotenone between the concentrations of 0.2 and 0.9 mg per liter. At lower concentrations the latter is increasingly more toxic, this being apparently the result of a lower threshold of toxicity. Its theoretical threshold is about half that of the active dihydrodeguelin.

Optically active dihydrodeguelin is more than twice as toxic as the inactive derivative—2.2 times as toxic according to a comparison of the minimum *ct* products. The theoretical threshold of toxicity of the inactive derivative is nearly twice as great as that of the active derivative. The minimum survival times, although apparently differing very little, occur in a region of much higher concentration (probably twice or more) in the case of the inactive derivative. However, despite the fact that the inactive form is nearly half as toxic as the levo form, it should not be inferred that the dextro twin of the latter would be nontoxic. The configuration of the compounds has not been established, and the possession of two asymmetric carbon atoms makes possible several optical isomers. Furthermore, the toxic action of the inactive derivative does not parallel that of a

[3] Powers used a instead of c_0 to indicate the threshold of toxicity concentration.

mixture half of which is the active derivative and half a nontoxic derivative. This is shown by replotting the survival-time curve, using half the actual concentrations of the determined points. At very low concentrations the resultant curve approaches very closely that of the active dihydrodeguelin but diverges at high concentrations.

Of interest also is the comparison of deguelin and the active dihydro derivative. According to the ratio of the minimum *ct* products, the latter is 1.33 times as toxic as the former. This may be compared with the value, 1.48, found for a similar structural relationship, in the case of four pairs of optically active rotenone derivatives (*6*). On the other hand, the inactive dihydro derivative is only 0.60 as toxic as inactive deguelin.

SUMMARY

A study has been made of the toxicity to goldfish of active and inactive dihydrodeguelins, and the results have been compared with each other and with those obtained with rotenone and deguelin. Three criteria were used as the basis of these comparisons—the maximum rate of increase of velocity with increase in concentration, $\tan \theta$; the minimum product of concentration and time $(ct)_m$; and Powers' formula, $\sqrt{\frac{\tan \theta}{c_0}}$, where c_0 is the theoretical threshold of toxicity. According to all these criteria the following descending order of toxicity was found: Rotenone, active dihydrodeguelin, deguelin, and inactive dihydrodeguelin.

Optically active dihydrodeguelin has essentially the same toxicity as rotenone between the concentrations 0.2 and 0.9 mg per liter. At lower concentrations rotenone is increasingly more toxic, approaching a maximum ratio of about 2 to 1.

Active dihydrodeguelin is more than twice as toxic as the inactive derivative. According to a comparison of the minimum *ct* products, the relative value is 2.2.

Active dihydrodeguelin is 1.33 times as toxic as inactive deguelin. This ratio is about the same as that (1.48) of the toxicities for active dihydro derivatives of rotenone to those of their parent compounds.

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A NEW BACTERIAL SPECIES ISOLATED FROM STRAWBERRIES[1]

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INTRODUCTION

In the course of a microbiological investigation of frozen-pack fruits and vegetables made in 1929 and 1930, the same species of bacterium was observed in great numbers in many samples of fresh strawberries going into frozen pack. Samples of berries were studied in 1929 from stations in Norfolk, Va., Fruitland, Md., and Selbyville, Del., and in 1930 at Portsmouth, Va. Plates from all four localities showed this bacterium to be the predominating organism in the fresh berries, whether or not they were washed before samples were taken. This organism occurs principally on the outside of the fruit and, so far as is known, has no bad effect upon the texture, flavor, appearance, or healthfulness of fresh or frozen strawberries.

The methods and media used in this study are those described in the manual of methods of the Society of American Bacteriologists.[2] One hundred grams of strawberries were placed in a sterile jar, and the berries were mashed thoroughly and mixed with 100 cc of sterile water. Plates were poured from this mixture after the proper dilutions had been made. Several colonies of the organism were selected for cultivation from plates poured in different localities, but as all the isolations reacted to the tests in a uniform way, only one will be described. Plates poured from the water before it was used to wash the berries and from the sugar used in the packs failed to show any colonies of the bacterium. This bacterium is believed to be a new species, and the name *Achromobacter delmarvae* is proposed for it.

DESCRIPTION

MORPHOLOGY

Achromobacter delmarvae, n. sp.

Short, medium-sized rod with rounded ends. Average size 1.5μ by 0.75μ. Occurs as single rod or in pairs, also in short chains. No capsules or spores demonstrated. Nonmotile, Gram-negative, not acid-fast. In old cultures a few rods found slightly larger than the ordinary vegetative size.

CULTURAL CHARACTERS

On beef-infusion agar, pH 7.0, colonies visible in 24 hours at 26° to 31° C. Develop at moderate rate, but always small, rarely over 5 mm in diameter, even when plates are scantily seeded. Colonies circular, raised, with smooth edges, glistening, translucent, bluish-white color in all lights. Internal structure amorphous, surface smooth. Buried colonies lens-shaped. Colonies on gelatin similar to those on agar.

Agar stroke cultures show abundant filiform growth, raised, glistening, smooth, translucent, bluish white, no odor; old cultures slightly viscid; medium unchanged.

Agar stab cultures grow abundantly. Surface growth round, smooth, glistening, bluish white, raised. Filiform growth the whole length of stab, but growth is best at top.

Gelatin stab cultures show scanty growth and no liquefaction after a month.

[1] Received for publication July 24, 1935; issued October 1935. Read at the annual meeting of the American Society of Bacteriologists, Baltimore, Md., December 31, 1931. Abstracted as follows: SMART, H. F. A NEW BACTERIAL SPECIES ISOLATED FROM STRAWBERRIES. Jour. Bact. 23: 41–42. 1932.

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Nutrient broth cultures show good clouding in 24 hours. Delicate white pellicle formed in 5 days. Precipitate abundant, white, and slightly stringy. No odor; color of medium unchanged.

Cultures on potato cylinders produce abundant growth, grayish white, glistening, smooth, raised. Medium changed from white to smoke-gray (Ridgway, pl. 46).[3]

PHYSIOLOGY

Optimum temperature for growth 26° C., good growth up to 31°. Growth at 37° very slight. Organisms remain alive at −8°; do not grow abundantly after being held at that temperature for any considerable length of time.

Optimum hydrogen-ion concentration about pH 7.0.

Bacterium white, but has slightly bluish color on agar and gelatin, probably caused by its translucent qualities. No color in broth, but on potato cylinders growth definite gray-white.

Indole not produced in tryptophane broth in 10 days.

Hydrogen sulphide and ammonia not produced in beef broth in 10 days.

Diastatic action of bacterium weak. Starch agar plates inoculated and tested with iodine solution; in 5 days very slight, clear zone of about 2 mm around the streak. Plates incubated for longer periods failed to show any wider clear zone.

Nitrates reduced in 7 days at 26° C. in medium containing 0.1 percent Difco peptone and 1 percent potassium nitrate.

Fermentation tubes used to study relation of organism to oxygen. Medium used was beef broth with 1 percent of the following sugars: Glucose, lactose, sucrose, mannitol, and glycerol. Growth in closed arm of all tubes but heavier in open end, showing bacterium to be facultative anaerobe.

Growth slow in sterile milk; acid curd formed in 12 to 14 days. Milk turned decided chocolate brown, beginning at top. Peptonization does not take place in 2 months.

Litmus in milk turns pink first and is entirely reduced in 5 days; pink color returns in 10 days, after which curd forms and browning of medium starts, as in the plain milk, in 12 to 14 days.

With bromcresol purple as indicator in fermentation tubes with beef-extract sugar broths, bacterium gives acid reaction after 5 days in glucose, lactose, glycerin, and mannite, and alkaline reaction in the same time in sucrose. No gas formed in any of the tubes.

SUMMARY

A bacterial species isolated from fresh strawberries is described.[4] This organism was found to be the predominating species in samples of berries collected from four localities in Delaware, Maryland, and Virginia over a period of 2 years. The species is thought to be one hitherto undescribed, and the name *Achromobacter delmarvae* is proposed.

The organism is a nonmotile, Gram-negative, nonspore-forming, short rod; average size, 1.5μ by 0.75μ; agar colonies are round, raised, glistening, translucent, bluish white, smooth, entire margin, amorphous; agar stroke cultures are filiform, raised, glistening, smooth, translucent, bluish white, no odor, medium unchanged, gelatin not liquefied; pellicle and stringy white precipitate in beef broth; grayish white on potato.

The organism grows best at 26° to 31° C.; is weakened at 37° and −8° C.; does not form indole, hydrogen sulphide, or ammonia; diastatic action is weak; nitrates are reduced; aerobic growth better than anaerobic; acid curd formed in 12 to 14 days; color of milk becomes chocolate-brown; reduction of litmus in 5 days in litmus milk with curd formation and browning as in plain milk; forms acid and no gas in glucose, lactose, glycerin, and mannite; alkaline reaction and no gas in sucrose.

[3] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912

[4] Other species of bacteria, yeasts, and molds isolated from fresh and frozen fruits and vegetables are listed in the following article: SMART, H. F. MICRO-ORGANISMS SURVIVING THE STORAGE PERIOD OF FROZEN PACK FRUITS AND VEGETABLES. Phytopathology 24: 1319–1331. 1934.

CHEMICAL COMPOSITION OF CANNED PEAS OF TWO VARIETIES OF DIFFERENT SIZES AND GRADES [1]

By W. S. THOMPSON, *junior chemist, Wisconsin Feed and Fertilizer Inspection Laboratory*, and W. H. PETERSON, *professor of agricultural chemistry, Wisconsin Agricultural Experiment Station*

INTRODUCTION

Canned peas, like other foods, are graded according to "quality", that is, flavor, tenderness, etc., rather than according to the nutritional value of their chemical constitutents. Chemical analysis is of prime importance, however, in supplementing the organoleptic tests by providing a sound basis for the selection of food from a nutritional point of view.

Data on the mineral composition of different commercial grades of peas are very inadequate. Previous investigations include papers by Diggs (*3*),[2] Boswell (*2*), and Sayre, Willaman, and Kertesz (*8*). These investigators studied the chemical changes in peas which are associated with the tenderness of the canned product, and reported that a high calcium content was associated with a tough pea. They found that potash fertilizers tended to reduce toughness and correspondingly to lower the calcium content. Conversely, calcium chloride applied to the soil made peas tougher. Percentages of dry matter, calcium, and total nitrogen were found to increase with size when calculated on the wet basis, but remained practically constant on the dry basis. In a later paper Kertesz (*6*) reported that the alcohol-insoluble solids (starch, dextrin, hemicelluloses, protein, and fiber) increase with maturity and hence serve as a good index of quality.

Because of the increased interest in the mineral content of foods, especially of the elements calcium, phosphorus, iron, copper, and manganese, it was considered desirable to obtain data on the variation in these elements in the commercial grades of canned peas. Data are also presented regarding the fiber, protein, and dry-matter content of the various sizes that are on the market.

MATERIAL AND METHODS

The canned peas used in this work were obtained from four different canneries, located in various parts of Wisconsin. By such a selection it was thought that the samples would be somewhat representative of the canned products of the State.

The can and contents were weighed, the liquor was drained off, and the can reweighed. The can was then emptied of the drained peas and weighed again. The liquor and drained peas were combined and dried in a steam oven in large evaporating dishes which were covered with heavy wrapping paper to prevent any material from dropping into the samples. They were then ground in an iron spice

[1] Received for publication Jan. 15, 1935; issued October 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 370.

mill to a fine powder.[3] From 5 to 13 cans of each size were treated in this way and a composite sample was formed by mixing equal portions of the individual samples. From this mixture aliquots were taken for the different determinations.

Nitrogen was determined by the Kjeldahl method (*1*) and calculated as protein N×6.25), phosphorus by the volumetric method of the Association of Official Agricultural Chemists (*1*), calcium by a modified McCrudden (*7*) method, copper by the method of Elvehjem and Lindow (*4*), manganese by the method of Skinner and Peterson (*9*), and iron by the method of Kennedy (*5*).

RESULTS AND DISCUSSION

Table 1 gives the variations in pea and liquor content per can and also the variations in the dry matter of the samples.

One hundred and twenty-seven samples, 52 of the Alaska or smooth variety, and 75 of the sweet or wrinkled variety, were used. The Alaska peas were of four different sizes, ranging from the smallest, no. 1, to the largest, no. 4. The sweet or wrinkled consisted of six different sizes ranging from no. 1 to no. 6 and an extra lot of ungraded peas of sizes 1 to 6. In any one size of canned peas there was found to be only a small variation in the weight of the total contents of similar cans, the maximum difference being 3.2 percent. However, there was considerable variation in the liquor and pea content of the various cans of each size, the maximum difference in weight of liquor being 19.5 percent and of the peas, 13.6 percent.

TABLE 1.—*Variation in quantity contained in can, and in dry matter of canned product, for Alaska and for an unknown sweet or wrinkled variety of peas, packed in no. 1 and no. 2 cans*

ALASKA

Pea size no.	Content	Weight of contents								Percentage of dry matter	
		Total		Drained peas		Liquor		Dry matter			
		No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*		
1	High	316	601	205	374	132	252	43	72	13.6	12.0
	Low	314	594	183	342	110	223	34	63	10.7	10.6
	Average [1]	315	597	197	361	118	236	38	68	12.1	11.4
2	High	323	604	215	400	120	223	48	103	14.9	17.0
	Low	319	597	200	381	104	204	41	84	12.8	13.9
	Average [1]	321	602	207	390	113	212	46	99	14.0	15.9
3	High	324	608	220	396	128	234	57	112	17.8	18.4
	Low	317	603	190	369	103	211	52	107	16.0	17.6
	Average [1]	320	606	207	380	113	226	54	110	16.8	18.0
4	High	325	607	211	410	118	226	62	123	19.0	20.4
	Low	321	596	204	370	114	193	59	105	18.1	17.5
	Average [1]	323	603	207	390	116	211	60	116	18.6	19.2

[1] Each figure is the average of 6 or 7 cans.

[3] The iron content of samples ground in a spice mill was from 22 to 65 percent higher than that of samples ground in a mortar. Only the latter method of grinding was used in the case of the iron analyses.

TABLE 1.—*Variation in quantity contained in can, and in dry matter of canned product, for Alaska and for an unknown sweet or wrinkled variety of peas, packed in no. 1 and no. 2 cans*—Continued

SWEET OR WRINKLED, VARIETY UNKNOWN

Pea size no.	Content	Weight of contents								Percentage of dry matter	
		Total		Drained peas		Liquor		Dry matter			
		No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can	No. 1 can	No. 2 can
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*		
	High	311	594	189	358	129	240	30	73	9.6	12.4
	Low	310	575	181	347	122	226	29	56	9.3	9.7
	Average [2]	311	587	185	353	126	235	30	63	9.4	10.7
	High	319	600	205	403	119	229	39	71	12.2	11.8
2	Low	313	582	199	353	111	194	36	56	11.4	9.6
	Average [2]	316	596	202	384	115	212	38	65	11.8	11.0
	High	324	602	212	400	124	231	46	81	14.2	13.5
3	Low	315	595	195	364	112	198	40	66	12.7	11.0
	Average [2]	320	599	202	384	118	214	43	76	13.3	12.7
	High	320	598	210	384	115	226	52	89	16.4	14.8
4	Low	316	586	205	360	108	214	49	76	15.3	12.9
	Average [2]	318	593	208	373	111	219	50	83	15.7	13.9
	High	325	599	205	386	127	230	55	103	16.9	17.2
5	Low	317	592	198	368	113	212	51	79	16.2	13.3
	Average [2]	320	596	202	377	118	219	53	96	16.5	16.0
	High	-------	608	-------	408	-------	239	-------	107	-------	17.8
6	Low	-------	595	-------	358	-------	195	-------	92	-------	15.3
	Average [2]	-------	601	-------	391	-------	210	-------	102	-------	17.0
	High	-------	603	-------	374	-------	230	-------	90	-------	14.9
Ungraded	Low	-------	576	-------	369	-------	203	-------	79	-------	13.7
	Average [2]	-------	592	-------	372	-------	218	-------	86	-------	14.5

Each figure is the average of 4, 7, or 9 cans, with the exception of size 1, no. 1 can (311 g), which includes only 2 samples.

Dry matter increased with size of pea. The percentage for the Alaska variety ranged from 11.4 for size 1 to 19.2 for size 4, and for the sweet or wrinkled from 10.7 for size 1 to 17.0 for size 4. This is in agreement with the work of Sayre, Willaman, and Kertesz (*8*).

It will be seen from figure 1 that on the wet basis the percentages of protein, calcium, phosphorus, and crude fiber increased with increase in size of pea. Also, that in each case the Alaska variety contained the higher percentage of each constituent. However, the increase of these constituents with size of pea is not preferential increase with respect to total solids; on the dry basis the percentage of these constituents remains practically constant, i. e., mineral elements and total solids are laid down at approximately the same rate. The data for protein and calcium in relation to size are in agreement with those reported by Sayre et al. (*8*).

Figure 2 shows the iron, copper, and manganese content of the peas calculated on the wet basis. The general trend was an increase with size, except in the case of copper in the Alaska variety; here there was a decrease. Again the composition of the Alaska variety was found to be different from that of the sweet or wrinkled variety.

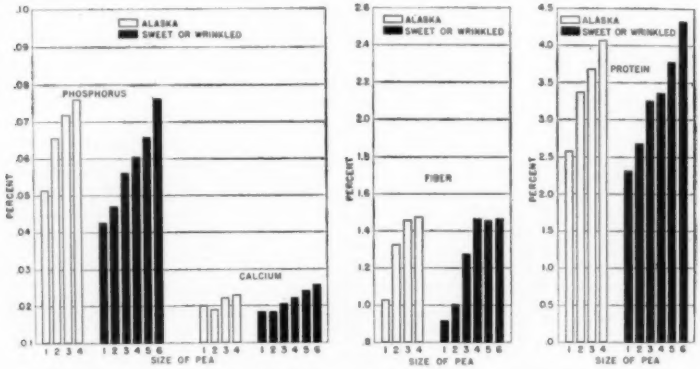

FIGURE 1.—Protein, calcium, phosphorus, and crude fiber content of canned peas (wet basis).

Because of a larger amount of dry matter and hence a larger sum of all the constituents, the larger pea has the greater food value.

The question arose as to whether such factors as soil, fertilizer treatment, inoculation, climatic conditions, etc., might have such an effect on the composition of the peas as so mask the variation due to size. In order to minimize these factors, samples were obtained from

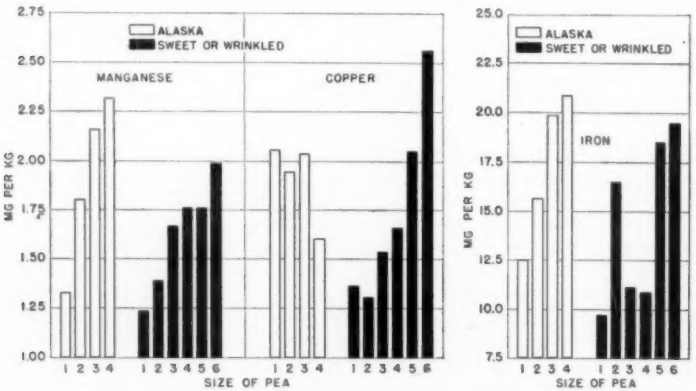

FIGURE 2.—Iron, copper, and manganese content of canned peas (wet basis).

a single cannery and their composition determined. In table 2 it is shown that the chemical composition of the peas from 1 cannery is very similar to that of the composite samples from 4 canneries. The variables, therefore, which affect the constituents of the canned product appear to have been practically the same for all the canneries.

TABLE 2.—*Protein, phosphorus, and calcium content of sweet or wrinkled and Alaska peas from 1 cannery and of composite samples from 4 canneries* [1] *(wet basis)*

Size no.	Variety and grade	Protein		Phosphorus		Calcium	
		A	B	A	B	A	B
	Sweet or wrinkled, unknown:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	Standard	2.22	2.43	0.0431	0.0418	0.0173	0.0202
2	Fancy	2.63	2.71	.0447	.0494	.0166	.0205
3	Extra standard	3.34	3.08	.0598	.0525	.0186	.0229
4	Standard	3.06	3.66	.0578	.0621	.0193	.0252
5	Do	3.96	3.63	.0721	.0595	.0228	.0249
6	Do	4.56	4.10	.0848	.0669	.0250	.0273
	Alaska:						
1	Extra standard	2.47	2.68	.0500	.0528	.0193	.0209
2	Standard	3.68	3.03	.0752	.0555	.0187	.0190
3	Do	3.83	3.49	.0799	.0639	.0234	.0212
4	Do	4.28	3.82	.0810	.0710	.0216	.0243

[1] A, figures for composite samples (5 cans each) from 1 cannery; B, figures for composite samples, (4 or 8 cans each) from 4 canneries.

As there are variations in quality within a given size of pea the question arose as to the relation between grade and composition when all samples were taken from one size. While there are differences in composition the figures in table 3 do not vary in any regular manner. In the Alaska variety the Standard grade contains the largest amount of dry matter, in the wrinkled variety the smallest amount. The protein content follows that of dry matter but the other constituents are quite constant.

TABLE 3.—*Variation in composition of Alaska and sweet or wrinkled peas of different grades (dry basis)*

Variety and grade [1]	Dry matter	Protein	Phosphorus	Calcium	Iron	Manganese	Copper
Alaska, size 2: [2]	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Standard	16.88	3.68	0.0752	0.0187	0.01010	0.00115	0.00125
Extra standard	16.06	3.08	.0561	.0210	.00943	.00126	.0010
Fancy	14.29	3.06	.0537	.0167	.01250	.00151	----------
Sweet or wrinkled, unknown, size 4: [2]							
Standard	13.37	2.98	.0542	.0216	----------	----------	----------
Extra standard	15.92	4.04	.0691	.0218	----------	----------	----------
Fancy	14.04	3.28	.0495	.0255	----------	----------	----------

[1] As given by the packer. [2] Average of 5 samples of each grade.

SUMMARY

The protein, calcium, phosphorus, copper, iron, manganese, and crude fiber content of canned peas of different sizes are given. The data are based on 127 samples from four canneries and represent the two important varieties of peas, smooth and wrinkled.

The percentages of dry matter, protein, calcium, phosphorus, copper, iron, manganese, and crude fiber of the canned product in general increased with size of pea. An exception is the copper content of the Alaska variety. If calculated on the dry basis the protein, calcium, and other constituents are found to be rather constant.

From certain points of view the nutritive value of the Alaska variety was superior to that of the sweet or wrinkled as the former contained more protein, calcium, and phosphorus.

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PUBESCENT AND GLABROUS CHARACTERS OF SOYBEANS AS RELATED TO RESISTANCE TO INJURY BY THE POTATO LEAF HOPPER [1]

By H. W. JOHNSON, *associate pathologist*, and E. A. HOLLOWELL, *agronomist*, *Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

Soybeans (*Soja max* (L.) Piper) may be divided roughly into the following three groups, on the basis of amount and type of pubescence on the leaves, stems, and pods: (1) Practically glabrous, (2) sparingly appressed-hairy, and (3) densely rough-hairy (fig. 1). The varieties grown commercially in the United States are practically all of the rough-hairy type, while many of the recent introductions from the Orient are glabrous or appressed-hairy. According to W. J. Morse,[3] these types, because of their resistance to the attack of a pod-boring insect, are widely grown in the Orient. When planted at the Arlington Experiment Farm, Rosslyn, Va., the glabrous type was heavily infested and seriously injured by the potato leaf hopper, *Empoasca fabae* (Harris); while rough-hairy varieties, grown nearby and presumably exposed to the same opportunities for infestation, were relatively free from leaf hoppers and from symptoms of leaf hopper injury. This difference is illustrated in figure 2.

Hollowell, Monteith, and Flint (*1*),[4] Pieters (*9*), and Monteith and Hollowell (*6*) have called attention to a similar situation in the case of red clover. The last-named writers report that—

> In red clover there is a direct correlation between hairiness of the leaves, petioles and stems and resistance to hopper injury. All the European and South American red clovers imported into this country (susceptible) are smooth or appressed pubescent, whereas red clover produced in this country and Canada (resistant) is rough pubescent.

Poos and Smith (*10*) compared the oviposition of *Empoasca fabae* females on red clover and soybean plants with the various types of pubescence and found that more nymphs hatched from the glabrous and appressed-hairy types than from the rough-hairy. The preference of the females for these types for oviposition is doubtless one reason for their greater injury when all three types are grown together in a comparative field test.

[1] Received for publication June 1, 1935; issued October 1935. This paper reports the results of certain phases of a cooperative study of the injury caused by *Empoasca fabae* (Harris) to forage legumes, which is being made at Arlington Experiment Farm, Rosslyn, Va., by the Division of Cereal and Forage Insects, Bureau of Entomology and Plant Quarantine, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Indebtedness is acknowledged to C. M. Woodworth, Illinois Agricultural Experiment Station, and W. J. Morse, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for supplying the seed used in these experiments; and also to F. W. Poos, Division of Cereal and Forage Insects, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for infesting field cages with *Empoasca*.

[3] Oral communication.

[4] Reference is made by number (italic) to Literature Cited, p. 380.

Two genetically distinct types of glabrousness have been demonstrated in soybeans: Nagai and Saito (*7*) in 1923 discovered a type in which glabrousness was dominant, while Stewart and Wentz (*12*) in 1926 reported a type in which glabrousness was recessive. In crosses with pubescent varieties, the ratio is a 3:1 in each case, show that a single-factor pair is involved. It appeared that a study of a large number of progenies from a cross between a pubescent and a

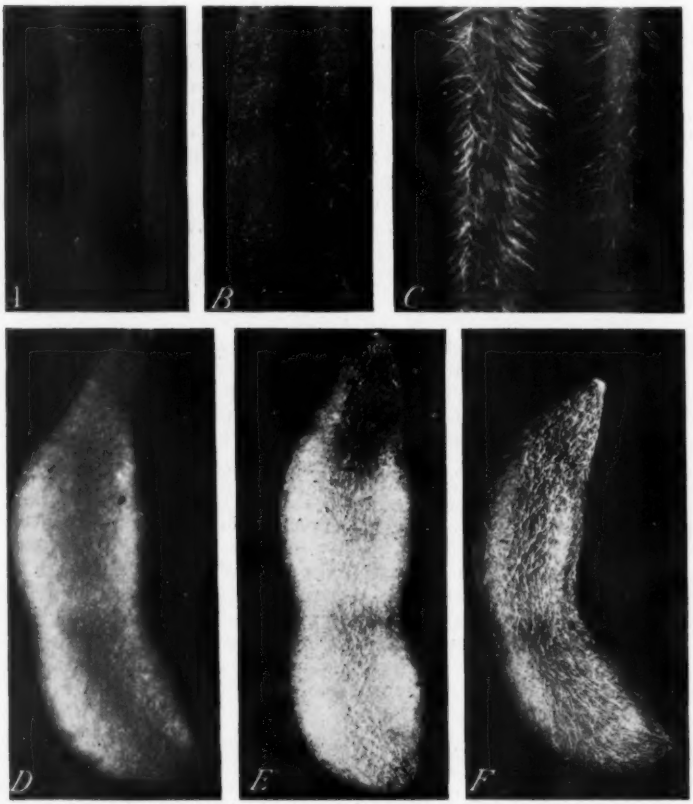

FIGURE 1.—Stems and petioles (*A–C*) and pods (*D–F*) of practically glabrous (*A, D*), sparingly appressed-hairy (*B, E*), and densely rough-hairy (*C, F*) soybeans. *A–C*, × 3; *D–F*, × 4.

glabrous soybean would yield evidence as to whether the rough-hairy pubescence or some other character of the pubescent plants was responsible for their resistance to injury by *Empoasca fabae*. Seed of 29 glabrous F_2 plants from a cross made between Illini (rough-hairy) and a dominant glabrous soybean were obtained from C. M. Woodworth of the Illinois Agricultural Experiment Station. This paper reports the results from a study of the F_3 progenies of these 29 plants grown at Arlington Experiment Farm in 1931, of 108 F_4

progenies grown in 1932, and of 73 F_5 progenies grown in 1933. Included also are observations on 34 glabrous and appressed-pubescent soybean introductions grown in 1931 and 32 similar introductions grown in 1932.

EXPERIMENTAL METHODS AND RESULTS

SOYBEAN PROGENIES

Seed of 29 glabrous F_2 plants from a cross between Illini (rough-hairy) and a dominant glabrous soybean was sown in progeny rows May 20, 1931. An excellent stand was secured and by July 13 marked differences in *Empoasca* infestation, stunting of growth, and injury to the leaves were evident between the glabrous and the rough-hairy individuals. The notes taken on this date may be summarized as follows: (1) Of the 29 F_3 progenies, 14 were homozygous glabrous

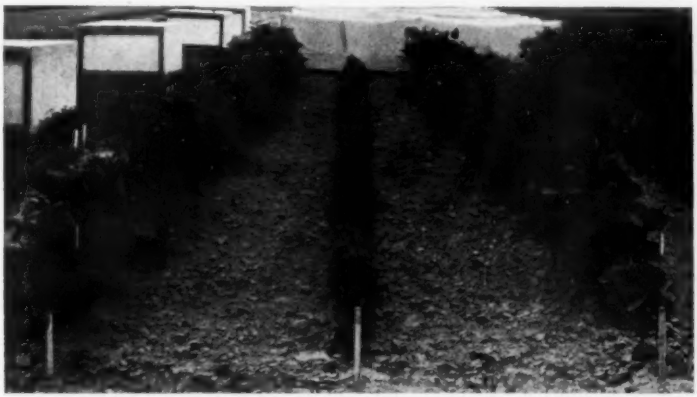

FIGURE 2.—Alternate rows of rough-hairy (Dixie) and glabrous (S. P. I.[4] no. 55069) soybeans growing at Arlington Experiment Farm on Aug. 6, 1931. The rough-hairy plants in the outside rows are practically free from *Empoasca* and show no symptoms of injury, while the glabrous plants in the center row are heavily infested and severely stunted and have curled leaves with yellowed necrotic margins.

and 15 segregated in a ratio of approximately 3 glabrous to 1 rough-hairy; (2) the glabrous individuals (total number 813), in both the homozygous and the heterozygous progenies, were all heavily infested with *Empoasca* and were severely stunted in growth, averaging only 8 inches in height, and their leaves were curled and had yellowed necrotic margins; (3) the rough-hairy individuals (total number 129) were all relatively free from *Empoasca* and averaged 15 to 16 inches in height, and their leaves showed no symptoms of leaf hopper injury.

These differences in degree of *Empoasca* infestation, stunting of growth, and leaf injury persisted throughout the season, and when final notes were taken on September 2 the glabrous plants averaged about 13 inches in height and bore relatively few seed pods, while the rough-hairy plants averaged about 25 inches in height and bore abundant seed. Late in September 1931, seed was harvested from individual glabrous and rough-hairy plants.

[4] S. P. I. denotes Seed and Plant Introduction, Bureau of Plant Industry.

Seed of 108 of the F_3 plants was sown in progeny rows on May 23, 1932, and, as in the previous season, by the middle of July marked differences in *Empoasca* infestation, stunting of growth, and injury to the leaves were evident between the glabrous and the rough-hairy individuals. The notes taken between July 19 and 27, 1932, may be summarized as follows: (1) Of the 108 F_4 progenies, 47 were homozygous glabrous, 32 segregated in a ratio of approximately 3 glabrous to 1 rough-hairy, and 29 were homozygous rough-hairy; (2) the glabrous individuals (total number 4,509), in both the homozygous glabrous and the heterozygous progenies, were all heavily infested with *Empoasca* and were stunted in growth, averaging about 15 inches in height, and their leaves were curled and were beginning to yellow at the margins, although little marginal necrosis had developed at this time; (3) the rough-hairy individuals (total number 5,449) were all relatively free from *Empoasca* and averaged 25 inches in height, and their leaves showed no symptoms of leaf hopper injury.

As the season advanced, marginal leaf necrosis became much more pronounced on the glabrous plants, and the differences in degree of *Empoasca* infestation and stunting of growth persisted. Late in September 1932, seed was harvested from individual plants in each progeny.

Seed of 73 of the F_4 plants was sown in progeny rows on May 24, 1933. By the middle of July, as in the two previous seasons, marked differences in *Empoasca* infestation, stunting of growth, and injury to the leaves were evident between the glabrous and the rough-hairy individuals. These differences, which are typical of those present in each of the three seasons, are shown in figure 3, illustrating the three types of F_5 progenies. As the season advanced the differences became even more pronounced, and the notes taken September 25, 1933, may be summarized as follows: (1) Of the 73 F_5 progenies, 32 were homozygous glabrous, 32 segregated in a ratio of approximately 3 glabrous to 1 rough-hairy, and 9 were homozygous rough-hairy; (2) the glabrous individuals (total number 3,248), in both the homozygous glabrous and the heterozygous progenies, were all infested with *Empoasca* and were stunted in growth, averaging about 14 inches in height, and their leaves were curled and had yellowed necrotic margins; (3) the rough-hairy individuals (total number 1,177) were all relatively free from *Empoasca* and averaged about 29 inches in height, and their leaves showed no symptoms of leaf hopper injury.

The data and notes on the three generations of progenies from a cross between Illini (rough-hairy) and a dominant glabrous soybean, which were grown in 1931, 1932, and 1933, are summarized in table 1. The consistency with which over a period of three generations in material of known genetic constitution the glabrous plants all were heavily infested and severely stunted and had curled hopper-burned leaves, while all the rough-hairy plants were practically free from *Empoasca*, grew vigorously, and had normal leaves with uninjured margins, appears to justify the conclusion that, in the soybeans tested, resistance to the injury caused by the potato leaf hopper was correlated with the occurrence of rough-hairy pubescence.

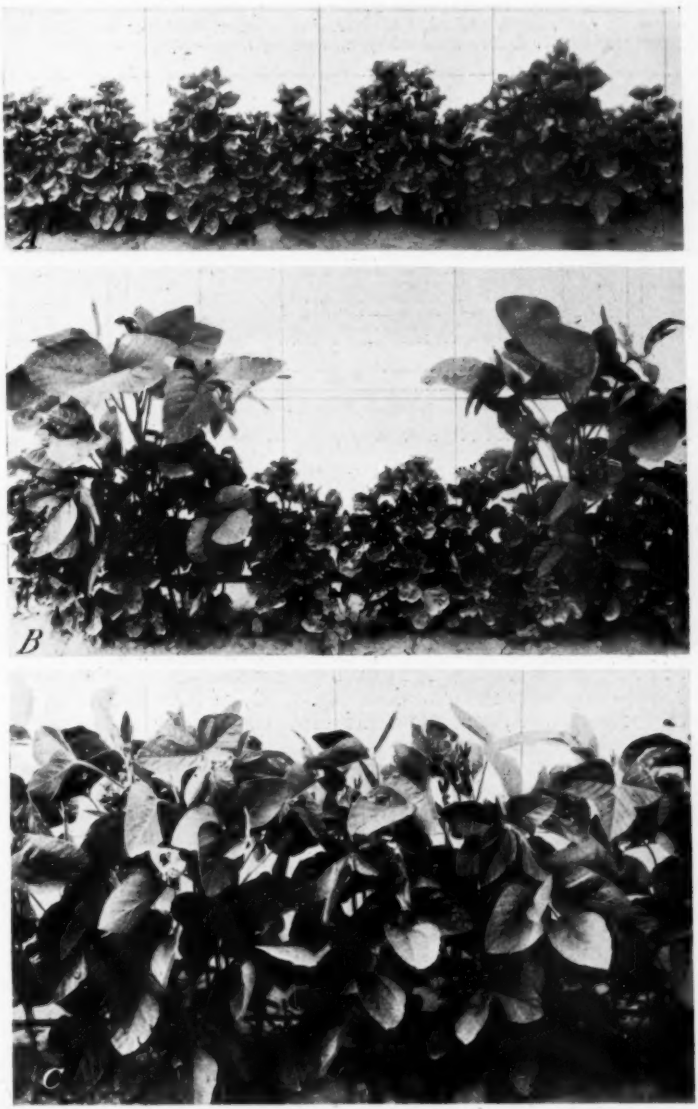

FIGURE 3.—Homozygous glabrous (*A*), heterozygous (*B*), and homozygous rough-hairy (*C*) F_3 progenies from a cross between Illini (rough-hairy) and a dominant glabrous soybean, growing at Arlington Experiment Farm on July 14, 1933. The glabrous plants are heavily infested with *Empoasca*, are severely stunted, and have curled leaves with yellowed necrotic margins; while the rough-hairy plants are practically free from leaf hoppers and are growing vigorously, and their leaves show no symptoms of hopper injury. The background is ruled in 1-foot squares.

TABLE 1.—*Summarized data on 3 generations of soybeans from a cross between Illini (rough-hairy) and a dominant glabrous type, grown at Arlington Experiment Farm, in 1931, 1932, and 1933 to determine whether resistance to injury by the potato leaf hopper is correlated with occurrence of rough-hairy pubescence*

Year	Generation	Phenotypes	Progenies	Individuals		
				Glabrous[1]	Rough-hairy[2]	Ratio of glabrous to rough-hairy
			Number	*Number*	*Number*	
1931	F_3	Glabrous	14	412	0	
		Glabrous and rough-hairy	15	401	129	3.1 : 1
1932	F_4	Glabrous	47	3,063	0	
		Glabrous and rough-hairy	32	1,416	442	3.2 : 1
		Rough-hairy	29	0	5,007	
1933	F_5	Glabrous	32	1,995	0	
		Glabrous and rough-hairy	32	1,253	422	2.97 : 1
		Rough-hairy	9	0	755	
		Total	210	8,570	6,755	

[1] Heavily infested with *Empoasca* and severely stunted, having curled leaves with yellowed necrotic margins.
[2] Practically free from *Empoasca*, vigorous, having normal leaves with uninjured margins.

SOYBEAN INTRODUCTIONS

Seven glabrous and 27 appressed-hairy soybean introductions were grown in rod rows adjacent to the F_3 progenies in 1931, while 16 glabrous and 16 appressed-hairy introductions were grown adjacent to the F_4 progenies in 1932. The rough-hairy varieties Herman and Dixie were included in the planting of both years for comparison. In both 1931 and 1932, the glabrous introductions (fig. 4, *A*) were heavily infested with *Empoasca*, were stunted in growth to about 1 foot in height, and had curled leaves with yellowed necrotic margins. The appressed-hairy introductions (fig. 4, *B*), on the other hand, were less heavily infested, and, while their leaves were crinkled and slightly yellowed at the margins, the plants attained a final height of 2 feet or more without evidence of marked stunting. The rough-hairy varieties attained a height of approximately 2½ feet each season and were practically free from leaf hoppers, and their leaves showed no symptoms of hopper injury.

Three of the glabrous introductions grown in 1931 and eight of those grown in 1932 contained a few rough-hairy plants, apparently as a result of segregation. In all such instances the glabrous plants were heavily infested and stunted while the rough-hairy plants were practically free from leaf hoppers and grew normally (fig. 4, *C*) just as in the case of the segregating progenies discussed above. The possibility of establishing leaf hopper resistant strains by selection of the hairy plants from these segregating introductions is evident. By this method, Kottur (*4*) and Kottur and Maralihalli (*5*) established a leaf hopper resistant strain of American cotton (Gadag No. 1) which almost entirely replaced the ordinary Dharwar-American variety (a mixture of hairy, sparingly hairy, and glabrous phenotypes) in the Dharwar-American tract in India.

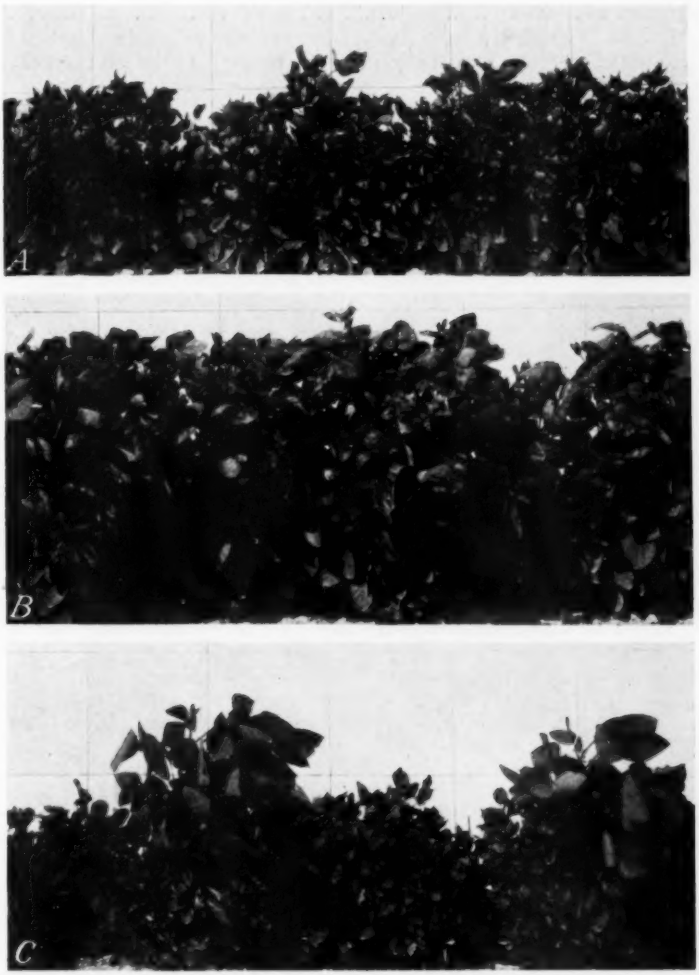

FIGURE 4.—Glabrous (*A*), appressed-hairy (*B*), and segregating (*C*) soybean introductions, growing on July 29, 1932. The glabrous plants have been stunted by a heavy infestation of leaf hoppers, the appressed-hairy plants are less heavily infested and show no marked stunting, although their leaves are crinkled and slightly yellowed at the margins. The rough-hairy plants in *C* are practically free from leaf hoppers, and their leaves show no symptoms of injury. The background is ruled in 1-foot squares.

DISCUSSION

Nagai and Saito (*7*), Stewart and Wentz (*12*), Owen (*8*), Woodworth and Veatch (*15*), and Veatch (*13*) have reported that glabrous soybeans were stunted in growth and yielded less than the pubescent varieties. Veatch (*13*) concluded from his genetic studies that in the dominant glabrous type of soybean, the factor P_1 inhibits the normal

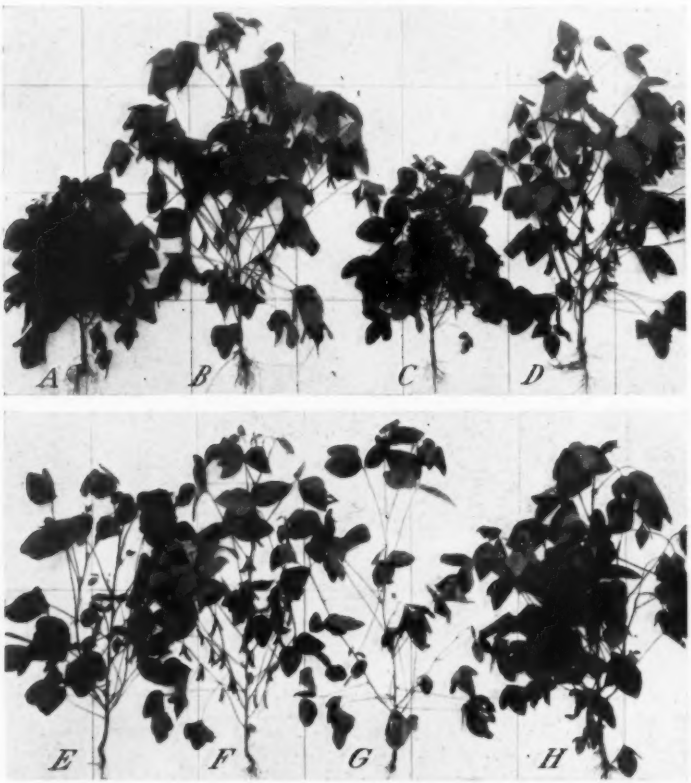

FIGURE 5.—Representative plants of three F_4 progenies from a cross between Illini (rough-hairy) and a dominant glabrous soybean, grown in a tobacco-cloth cage infested with *Empoasca* (*A–D*) and in a similar uninfested cage (*E–H*). The infested glabrous plants (homozygous, *A;* heterozygous, *C*) are stunted and have curled leaves with yellowed margins, while those kept free from leaf hoppers (homozygous, *E;* heterozygous, *G*) have uninjured leaves and have made practically as tall growth as the rough-hairy plants (homozygous, *B, F;* heterozygous, *D, H*). The background is ruled in 1-foot squares.

vigor and plant development as well as pubescence or is closely linked with factors that reduce vigor and development. Since P_1 is not present in the recessive glabrous type, Woodworth (*14*) concludes that here lack of vigor most likely is due to the recessive factor p_2, which is responsible for glabrousness in this strain. The writers have observed that when progenies are grown to the young plant stage in a greenhouse free from leaf hoppers, the rough-hairy seedlings grow

more rapidly and somewhat more vigorously than the glabrous seedlings, apparently because of some genetic difference. However, when progenies are grown to maturity out of doors, but under cages so that the plants are kept practically free from leaf hoppers, the glabrous plants, while much lower in seed yield, are not severely stunted in growth. This fact is illustrated by figure 5, showing plants from the F_4 generation of the cross between Illini (rough-hairy) and a dominant glabrous soybean, which were grown in tobacco-cloth cages in 1932. The plants above (*A–D*) are from a cage containing half of each of three phenotypically different progenies, which were artificially infested with potato leaf hoppers. The plants below (*E–H*) are from a cage containing the other halves of the same progenies, which were kept free from leaf hoppers throughout the season. The infested glabrous plants (*A*, *C*) are stunted and have curled leaves with yellowed margins, while those kept free from leaf hoppers (*E*, *G*) have uninjured leaves and are practically as tall as the rough-hairy plants. It would appear, therefore, that the extreme differences in growth and vigor between the rough-hairy and glabrous types when grown in the field are due in part to infestation of the glabrous plants with potato leaf hoppers.

The significance of the dense rough-hairy pubescence in the resistance of this type to leaf hopper injury is an interesting question which so far has been answered only in part. Monteith and Hollowell (*6*) observed that plants with succulent and fleshy veins or petioles, such as cowpeas, seem to be preferred by the hoppers, and conclude that, although dense pubescence evidently serves as a mechanical obstruction which in some way interferes with the activities of the insects, there appears to be some factor involved other than or in addition to that of hairiness. Poos and Smith (*10*) compared oviposition and nymphal development of *Empoasca fabae* on plants with the various types of pubescence and concluded that factors other than amount and type of pubescence probably are responsible, at least in part, for the resistance to injury by this leaf hopper observed in some of the strongly pubescent varieties of legumes. Jewett (*2*) observed no very direct relation between amount of injury and amount of pubescence in red clover and considered some unknown factor to be responsible for the resistance of the native rough-hairy type. He later (*3*) reported that leaves of Kentucky red clover (rough-hairy) were more resistant to puncturing than leaves of Italian red clover (practically glabrous) and concluded that the greater resistance of Kentucky clover to injury by *E. fabae* is due, in part at least, to greater resistance to puncturing. Since the histological work of Smith and Poos (*11*) showed that *E. fabae* feeds of necessity upon the vascular tissues of its hosts, measurements of the resistance of the leaf blades of red clover to mechanical puncturing probably are of only slight value in explaining differences in resistance of strains with the various types of pubescence. The results reported in this paper suggest that in the soybeans tested the mechanical protection of dense rough-hairy pubescence was primarily responsible for the resistance of the hairy plants to injury. Otherwise, one must assume the existence of some other character the inheritance of which is controlled by the same hereditary complex as pubescence. If another character for resistance existed, its presence probably would have become evident in at least a few of the segregating progenies.

SUMMARY

Soybean progenies of the F_3, F_4, and F_5 generations from a cross between Illini (rough-hairy) and a dominant glabrous soybean were grown in 1931, 1932, and 1933 to determine whether the resistance of rough-hairy soybeans to the injury caused by the potato leaf hopper, *Empoasca fabae* (Harris), is correlated with the occurrence of rough-hairy pubescence in material of known genetic constitution.

In the three generations tested the glabrous individuals of both the homozygous glabrous and the heterozygous progenies were all heavily infested with *Empoasca*, severely stunted in growth, and had curled leaves with yellowed necrotic margins. The rough-hairy individuals, on the other hand, were almost entirely free from *Empoasca*, and grew vigorously, and their leaves showed no symptoms of leaf hopper injury.

Glabrous and appressed-hairy soybean introductions from the Orient were grown adjacent to the progenies in 1931 and 1932. The glabrous plants were heavily infested with leaf hoppers and were severely stunted, while the appressed-hairy plants were less heavily infested and were not markedly stunted in growth, although many of their leaves were crinkled and had yellowed margins which later became necrotic. Some of the glabrous introductions contained rough-hairy plants, probably segregates, which were practically free from leaf hoppers and showed no symptoms of injury.

It would appear that in the soybeans tested resistance to leaf hopper injury was due to the rough-hairy pubescence or to some character the inheritance of which is controlled by the same hereditary complex as pubescence. No evidence of the existence of such a character was obtained in these studies, which involve three generations of progenies consisting of 8,570-glabrous and 6,755 rough-hairy individuals.

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